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QUENCHING OF THE SYSTEM II CHLOROPHYLL FLUORESCENCE BY THE PLASTOQUINONE POOL

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Summary

If 3-(3,4-dichlorophenyl)-1,1-dimethylurea is added to dark adapted chloroplasts, the maximum fluorescence F_{stat} when Q is completely reduced, is lower than the maximum fluorescence reached with no 3-(3,4-dichlorophenyl)-1,1-dimethylurea present during a continuous illumination F_p. If 3-(3,4-dichlorophenyl)-1,1-dimethylurea is added during illumination a quenching develops and the fluorescence drops from F_p to F_{stat}.

A study was made of that quenching and we show that it corresponds to a non-photochemical quenching by the oxidized pool of plastoquinones A:

1. When 3-(3,4-dichlorophenyl)-1,1-dimethylurea is added during illumination, A initially reduced is reoxidized by System I. The rate of the fluorescence quenching which develops upon addition of 3-(3,4-dichlorophyl)-1,1-dimethylurea is also dependent on System I activity.

2. If A is reduced by a strong illumination, it is slowly reoxidized in the dark by oxygen. The maximum fluorescence level F_t reached during an illumination following 3-(3,4-dichlorophenyl)-1,1-dimethylurea addition is related to the oxidation level of A.

3. In low light intensity, the amount of reduced plastoquinone is increasing with increasing $MgCl_2$. The quenching observed also depends on $MgCl_2$ concentration.

4. If A is maintained reduced by dithionite, the quenching is abolished.

This quenching exerted by the oxidized plastoquinones is 20% of the maximum fluorescence. It is weak compared to the very different photochemical quenching of open Photosystem II centers.

Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Introduction

In higher plants and algae there exists a variable fluorescence emitted by Photosystem II. It has been shown that the fluorescence intensity and the photochemical rate vary in a complementary way showing that the deactivation of excited chlorophyll by photochemistry is the main competitor of deactivation by fluorescence. In other words, the fluorescence is modulated by the oxido-reduction state of the primary acceptor Q [1].

However, several other non-photochemical quenchings have been shown to exist: Delosme introduced the concept of a non-photochemical quencher R kinetically homogeneous with the plastoquinone pool A [2]. Joliot et al. showed the existence of long lifetime fluorescence states correlated to the S_2 and S_3 states of System II [3]. Murata showed that F_{max} (in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was considerably increased by the addition of Mg^{2+}. The explanation of the effect was a decrease in the rate of exciton spill-over from System II to System I, that is a change in non-radiative deactivation pathway in competition with the fluorescence emission [4].

Wraight et al. [5] have observed a difference in the maximum fluorescence, when Q is reduced, in presence and absence of DCMU; they suspect an effect of the plastoquinone pool. Jennings and Forti observed a quenching developing upon addition of DCMU in the light, and studied the effects of cations on that quenching [6].

In the present work, we will attempt to prove that the non-photochemical quencher R is identical to the oxidized plastoquinone pool, and is the origin of the quenching observed in presence of DCMU. This hypothesis allows a common explanation for several of the quenchings described above.

Materials and Methods

Materials

Pea chloroplasts preparation. The chloroplasts are type II chloroplasts without external membranes.

The grinding is done in 0.4 M sorbitol, 0.1 M Tricine (pH 7.8), bovine serum albumin at 2 mg/ml. After filtration and centrifugation the pellet is washed in 10 mM NaCl. After a second centrifugation the chloroplasts are suspended in 0.4 M sorbitol, 10 mM Tricine (pH 7.8), 10 mM NaCl, 10 mM $MgCl_2$, at a concentration of 1—4 mg chlorophyll per ml.

The experiments are done at room temperature at a chlorophyll concentration of 10 μg/ml in the above described buffer, without $MgCl_2$ if mentioned, and in the presence of 10^{-6} M gramicidin to avoid the field and pH effects. The chloroplasts are dark adapted for 10 min before addition of chemicals or illumination.

Chlamydomonas mutant Fl_5. This mutant, isolated by J. Garnier, is devoid of *P*-700 [7].

Fluorescence measurements. The sample cuvette is 10 X 10 X 30 mm, stirred by magnetic stirring. The fluorescence is excited by a xenon lamp through a monochromator Bausch and Lomb high intensity and a 4-96 corning filter. The

exciting light intensity can be varied with neutral filters. The fluorescence is detected in the red through a light guide and a 2-64 corning filter plus a wratten 90 filter by a photomultiplier EMI with a S20 cathode.

The fluorescence kinetics recording is done either directly by a recorder with a response time of 0.15 s or through a multichannel analyzer SEIN which allows integration of the curves. The response time is then 6 μs per channel.

Results

When DCMU is added in the dark prior to illumination the maximum fluorescence level (F_{st}) obtained is depicted in Fig. 1A.

Fig. 1B shows the fluorescence obtained during a strong illumination (100 photons/center per s) of 40 s (level F_p). Addition of DCMU during illumination produces a level F_M close to F_p followed by a fluorescence decrease, the fluorescence finally reaching a F stationary (F_{st}) identical to that of Fig. 1A. If DCMU is added in the dark after 40 s preillumination, the fluorescence kinetics during a second illumination starts from F_t lower than F_M and always reached the same F_{st} value (Fig. 1C).

We tentatively correlate the different fluorescence levels F_{st}, F_p, F_M, F_t to different oxido-reduction states of the plastoquinone pool A which is reduced in F_p and F_m, oxidized in F_{st} and in an intermediary state for F_t.

The following experiments were undertaken to prove this point:

(a) In the presence of DCMU the reoxidation rate of A during an illumination is dependent on System I activity. It can be varied by changes in the light intensity or by the addition of an exogenous System I acceptor. Table I shows

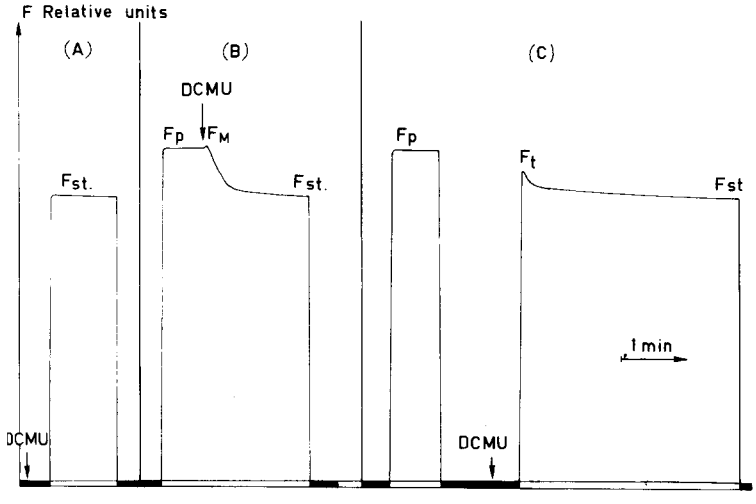

Fig. 1. The intensity of fluorescence in arbitrary units is plotted as a function of time. Chloroplasts are dark adapted diluted in a buffer at pH 7.8 with $MgCl_2$ and gramicidin at a chlorophyll concentration of 10 μg/ml. Fluorescence is excited in the blue and detected in the red. The light intensity is around 100 photons/center. second. A, DCMU 10^{-5} M is added to dark adapted chloroplasts before the illumination. B, DCMU is added after 40 s of illumination. C, DCMU is added in the dark after a 40 s illumination and prior to a second illumination.

TABLE I

KINETICS OF THE QUENCHING DEVELOPED UPON DCMU ADDITION IN THE LIGHT

Light intensity (photon/center per s)	Additions	Time to develop half quenching (s)
Sample 1		
6	DCMU 10^{-5} M	6.0
20	DCMU 10^{-5} M	5.0
40	DCMU 10^{-5} M	3.9
100	DCMU 10^{-5} M	3.3
Sample 2		
230	DCMU	2.6
230	DCMU + methylviologen $2.5 \cdot 10^{-6}$ M	0.3

that indeed the rate of fluorescence quenching depends on the light intensity. In the presence of methylviologen this rate is much faster than in DCMU alone.

(b) If one assumes that the intensity of fluorescence F and the photochemical rate are linearly related to each other in a complementary fashion [8], it is possible to quantitatively evaluate the pool size of oxidized plastoquinone by the complementary area of the fluorescence rise curve [9,10]. In darkness, A is reoxidized by the oxygen in the air. These reoxidation kinetics can be followed by measuring the complementary area of the fluorescence induction with no DCMU present and after different dark periods following a 30 s strong illumination which allows a complete reduction of A.

After the same dark periods we have detected the maximum fluorescence level reached in an illumination following DCMU addition. (Measurement of F_t as in Fig. 1C). When plotting the fluorescence quenching: $(F_M/F_t - 1)$, F_M being the fluorescence level when DCMU is added in the light, versus the oxida-

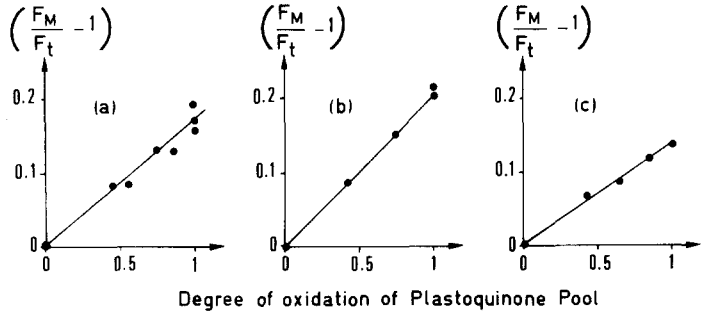

Fig. 2. F_M and F_t being the fluorescence levels as defined in Fig. 1 $F_M/F_t - 1$ is plotted versus the degree of oxidation of the plastoquinone pool. The amount of oxidized plastoquinone is measured by the complementary area of a fluorescence rise curve during a second illumination. The implicite variable is the dark time evolved between the end of the first illumination and beginning of the second illumination. a corresponds to normal chloroplasts; b corresponds to chloroplasts incubated for 15 min in the dark with 10^{-2} M hydroxylamine. c corresponds to the *Chlamydomonas* mutant $F1_5$ devoided of System I centers; other conditions are described in Fig. 1.

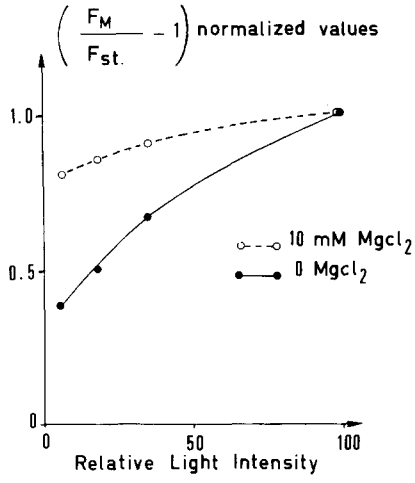

Fig. 3. Effect of the light intensity on the fluorescence quenching developed upon addition of DCMU during illumination in the presence or absence of $MgCl_2$. F_M and F_{st} are measured as in Fig. 1B. Because of an increase in spill over from System II to I in the absence of $MgCl_2$, the F_M observed in the absence of $MgCl_2$ is half that observed in the presence of 10 mM $MgCl_2$. For a better comparison, we normalized ($F_M/F_{st} - 1$) observed at maximum light intensity to 1 both in the presence and the absence of $MgCl_2$. Other conditions are described in Fig. 1.

tion level of A (as measured by the complementary area), we obtain a linear relationship that is a Stern-Volmer type of quenching (Fig. 2A).

(c) As stated in the introduction Jennings and Forti [6] had observed the quenching of fluorescence produced by the addition of DCMU during an illumination. This quenching was dependent of $MgCl_2$ concentration. On the other hand Marsho and Kok [11] have shown that under non-saturating light intensities the amount of reduced plastoquinones was increased with increasing concentration of $MgCl_2$, because of a change in the equilibrium constant between the two Photosystems. 10 mM $MgCl_2$ promotes System II activity, no $MgCl_2$ promotes System I activity. Under strong light intensity, the plastoquinones are completely reduced whatever the cation concentration.

Therefore, we measured the fluorescence quenching produced by addition of DCMU in the light, with or without 10 mM $MgCl_2$ and at different light intensities. Fig. 3 shows clearly that the light intensity influences the amplitude of the quenching. In low light, the degree of reduction of the plastoquinone is lower than in high light, especially in the case of no Mg^{2+}. Therefore upon addition of DCMU the apparent quenching is much lower. When light intensity is maximum, the plastoquinone pool is reduced in both conditions, and we have a maximum quenching equal to 0.20 for Mg^{2+} and 0.10 in the absence of Mg^{2+}, this difference being explained by the presence of spill-over in absence of Mg^{2+}, which competes with the other ways of deactivation, according to Murata [9] and Briantais et al. [12].

(d) The plastoquinone pool can also be reduced by addition of a strong reductant: 3 mM sodium dithionite. It reduces the plastoquinone pool in the dark [13].

One way to show its effect is to first add DCMU during a strong illumination,

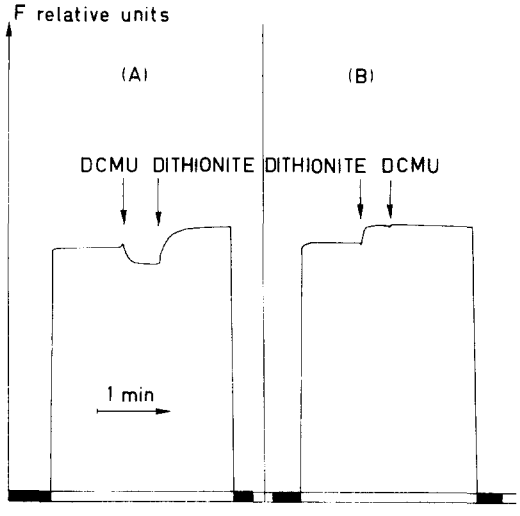

Fig. 4. Effects of DCMU and dithionite on fluorescence during a strong illumination. (A): DCMU 10^{-5} M is added after 1 min strong illumination, then 3 mM $Na_2S_2O_4$ is added when F_{stat} is reached. (B): 3 mM $Na_2S_2O_4$ is added during illumination prior to the addition of 10^{-5} M DCMU. Other conditions are described in Fig. 1.

wait for the complete development of fluorescence quenching (F_{st} level) then add dithionite which reduces the plastoquinone pool. In that case the quenching is reversed as shown in Fig. 4A.

Another way to show its effect is to add ditionite during the strong illumination prior to the addition of DCMU. In that case no fluorescence quenching is produced by the addition of DCMU since the plastoquinone pool is maintained in its reduced state by dithionite. The maximum fluorescence observed with dithionite is slightly higher than F_p or F_M. This might be explained by the suppression of a fluorescence quenching by oxygen since dithionite produces a good anaerobiosis.

Joliot and Joliot have also observed this fluorescence increase [14]. As already state by these authors when dark adapted chloroplasts are incubated in the dark with DCMU alone first, then dithionite, the addition of dithionite has only a small effect on F_o.

If we compare the effects of the addition in the dark of DCMU alone or DCMU followed by dithionite, we observe when dithionite is present a slight increase of F_o (5—11%) and a larger increase of the maximum level (20—30%) (Table II). The complementary areas in the two conditions are identical: this means that the concentration of the primary electron acceptor Q is the same and that the quenching destroyed by dithionite is a non-photochemical quenching. If dithionite is added before DCMU, the maximum level of fluorescence is the same as with DCMU before dithionite, but F_o level is higher and the complementary area is much smaller.

(e) It has been shown that the donor side of Photosystem II has an influence on the fluorescence, the higher S states corresponding to a higher F_o level [3]. To make sure that the quenching we are dealing with is not due to the oxida-

TABLE II

SUPPRESSION OF THE QUENCHING BY OXIDIZED PLASTOQUINONES IN F_0 AND F_{max} BY DITHIONITE ADDITION

Addition (in darkness before illumination)	Fluorescence (relative units)		Complementary area F_{max}
	F_0	F_{max}	
DCMU	1 ± 0.03	2.85 ± 0.05	1.00 ± 0.05
DCMU (15 s) then dithionite (15 s)	1.08 ± 0.03	3.55 ± 0.05	1.00 ± 0.05
Dithionite (15 s) then DCMU (15 s)	1.50 ± 0.03	3.55 ± 0.05	0.50 ± 15

tion state of the donor side, we repeated the experiments with chloroplasts in which the oxygen evolving system has been destroyed by hydroxylamine which then serves as an exogenous donor to Photosystem II in a one quantum process [15,16]. For that purpose the chloroplasts were dark incubated during 15 min with 10^{-2} M NH_2OH. The observed phenomena are similar, the relationship between the fluorescence quenching and the amount of oxidized plastoquinone pool is shown in Fig. 2B.

(f) In order to avoid any artifact being due to some solubilization of plastoquinone during chloroplasts extraction, we also repeated the experiments with whole algae. We choose a mutant devoid of System I activity so that a complete reduction of plastoquinones can be produced by illumination; the reoxidation being achieved by the ambiant oxygen. We again observed the same phenomenon, the linear relationship between the fluorescence quenching and the amount of oxidized plastoquinone pool is shown in Fig. 2C.

Discussion

We have shown that apart from the photochemical quenching produced by the competition between photochemical activity, i.e. reduction of the primary acceptor Q and fluorescence, there exists a non-photochemical quenching of the variable fluorescence.

The quencher described in this paper was shown to correspond to the oxidized plastoquinone pool A. It is identical to the quenching first introduced by Delosme as non-photochemical quencher R to explain the non-photochemical quenching remaining under high intensity illumination when all Q is in its reduced state [2]. This non-photochemical quenching was destroyed during prolonged illlumination and shown to be kinetically analogous to the reduction of the plastoquinone pool.

It is well known that in vitro the fluorescence of chlorophyll solutions is quenched by the oxidized form of various quinones [17,18]. In vivo exogenous quinones, DBMIB or benzoquinone quench fluorescence in their oxidized form and are no longer quenchers when reduced. Also, van Gorkom in his deoxycholate particles observed an important quenching by plastoquinone [19].

The more pronounced quenching of F_{max} (20%) as compared to that of F_o (5—11%) by the oxidized plastoquinone pool is a trivial effect if one considers that the situation in vivo is comparable to that of a Stern-Volmer quenching in

solution because of the existence of an efficient exciton transfer between chlorophylls. With this assumption the fluorescence yield is inversely proportional to the sum of the rate constants of various deactivation pathways [23]. This in turn implies that the relative modulatory effect of a change in the rate constants of any pathway is proportional to the fluorescence yield.

The observed non-photochemical quenching is independent of the donor side of System II: it exists in chloroplasts with or without hydroxylamine washing. It is not due to a direct effect of the cation concentration. In high light intensities the maximum fluorescence yield is lower in the absence of Mg^{2+} than with Mg^{2+} present because of the additional deactivation pathway due to spillover from System II to System I [9,12]. Therefore the quenching by oxidized plastoquinone is lower in the absence of Mg^{2+}. Under low light intensities, the reduction level of A is dependent on the presence of cations, as shown by Marsho and Kok [11], and this produces an additional modulation of the fluorescence yield.

Bocquet [21], Velthuys and Amesz [22] have shown the existence of a carrier B wich exchanges electrons one by one with Q, two by two with A. One problem is to know whether B partipate to the non-photochemical quenching. DCMU is supposed to prevent the electron flow between Q and A by lowering the mid-point potential of B; therefore, when DCMU is present, Q is easily reduced by B^-. The study of the combined effects of dithionite and DCMU (Table II and ref. 14) shows that if DCMU is added prior to dithionite, it prevents the reduction of Q by dithionite (the complementary areas of the fluorescence inductions are equivalent with DCMU alone, or DCMU and dithionite added after). We suppose that it means that B cannot be reduced by dithionite when DCMU is present. The maximum fluorescence level when dithionite is added before DCMU (that is when both B and A are reduced) is equal to the fluorescence level when DCMU is added first (that is when only A is reduced). Consequently, we concluded that the non-photochemical quenching is essentially due to A.

In conclusion we have proved by several ways that when in its oxidized form the endogenous pool of plastoquinones, located between the two Photosystems, exerts a non-photochemical quenching on the variable fluorescence of Photosystem II. This quenching is weak if compared to the very efficient photochemical quenching exerted by an open center, i.e. an oxidized primary electron acceptor Q.

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