Biochimica et Biophysica Acta 808 (1985) 103–111
Elsevier

BBA 41751

Effects of herbicides on binary oscillations of ultraviolet flash-induced absorption changes in chloroplasts

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(Received December 7th, 1984)

Key words: Electron transfer; Plastoquinone; Herbicide; Photosystem II; (Spinach, Chenopod chloroplast)

The competition between various herbicides and plastoquinone, at the Q_B site of Photosystem II, has been studied by measuring absorption changes between 300 and 360 nm in spinach and chenopod chloroplasts, in response to a train of saturating short xenon flashes. A complex pattern was observable without addition of chemicals interfering with electron flow. The effect of potassium ferricyanide, of hydroxylamine and of valinomycine-K^+ permitted to simplify the pattern and, in particular, to observe binary oscillations with flash number, attributable to the functioning of the two-electron gate Q_B. Herbicides belonging to different classes block electron transfer when added at high concentration. At low concentration, however, inverted binary oscillations become observable. When the chloroplasts have been first oxidized with ferricyanide, this behaviour develops progressively in response to illumination. Varying the herbicide concentration, it appears that the concentration inducing maximum binary oscillations correlates with inhibition of linear electron transfer, within each class of herbicides. Phenolic herbicides induce the largest oscillations and ureas the smallest. The binary oscillations have the spectrum of the plastoquinone anion. The results clearly show that the studied herbicides compete efficiently with Q_B, but not with Q_B^-, at the Q_B binding site. Atrazine-resistant chenopod chloroplasts still display normal binary oscillations in the absence of herbicide, or in the presence of atrazine alone. They are highly sensitive to DCMU and to *i*-dinoseb, but no inverted binary oscillations could be observed with these herbicides.

Introduction

The primary photoreactions of Photosystem II (PS II), in oxygen-evolving systems, lead to the reduction of a reaction center-bound plastoquinone Q_A. Under physiological conditions, Q_A can accept only one electron. It then reduces a secondary plastoquinone Q_B which appears to be functionally different from Q_A in two respects. First, it can accept sequentially two electrons, leading to the fully reduced quinol: Q_B is thus considered as a 'two-electron gate' (reviewed in Ref. 1). Second, according to current hypotheses [2–6], its binding to the reaction center depends on its reduction state: it would be firmly bound to a reaction center protein of 32 kDa (sometimes named Q_B protein) in state Q_B^-, and bind more loosely when it is oxidized (Q_B) or fully reduced. Q_B is probably anyone of the molecules of a pool of plastoquinones, which functions as Q_B when it is bound to its apoprotein.

Abbreviations: ΔA, absorption change; PS II, Photosystem II; CMU, 3-(*p*-chlorophenyl)-1,1-dimethylurea; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea; *i*-dinoseb, 2,4-dinitro-6-*iso*-butylphenol; DNP-INT, 4-dinitrophenylether of 2-iodo-4-nitrothymol; DCIP, 2,4-dichlorophenol-indophenol; INT, 2-iodo-4-nitrothymol; DNOC, 4,6-dinitro-*o*-cresol.

In dark-adapted chloroplasts, about two third of Q_B is in the oxidized state [7,8]. Upon excitation by a sequence of flashes which induce only one turn-over of the PS II center, the state of reduction of components belonging to the electron-transfer path between PS II and PS I oscillates with a period of 2 with flash number [7,9]. This has particularly been shown for the couple (Q_B, Q_B^-), by flash absorption spectroscopy, taking advantage of the absorption band of the plastoquinone radical anion around 320 nm [8,10,11].

Many commonly used herbicides inhibit the reoxidation of Q_A^- [12]. According to current hypotheses, these herbicides exert their inhibitory effect primarily by binding at the site of Q_B, or at a neighbour site, thus preventing the binding of Q_B [3,4,6]. Chemical experiments on the competitive binding of quinones and herbicides offer a good support to that hypothesis [13–16]. In this work we have studied the effect of different herbicides, and of their concentration, on flash-induced absorption changes due to the plastoquinone radical-anion (Q_A^- or Q_B^-) in chloroplasts. Some treatments have been used to control the redox state of Q_B before the train of flashes, or to suppress the absorption changes due to the oxygen-evolving system. Inverse oscillations were observed in the presence of herbicides, and attributed to the successive disappearance and regeneration of Q_B^-, showing that active PS II centers were enriched in state Q_B^-. These effects have also been studied in chloroplasts from chenopod plants which are resistant to the herbicide atrazine, with the surprising result that no inverse oscillations could be observed, with any herbicide concentration, although electron transfer appears to function normally.

Material and Methods

Biological Material

Spinach plants were cultivated in a field. Atrazine-susceptible and atrazine-resistant biotypes of chenopod (*Chenopodium album*), collected near Dijon (France) were grown under controlled conditions with 16 h photoperiod (at the Phytotron, CNRS, Gif-sur-Yvette). The chenopod seeds were kindly provided by Drs. El Anouar and Ducruet (INRA). To prepare the chloroplasts, 20–50 g of leaves were ground for 5 s in 150–350 ml of isolation medium (350 mM sorbitol/20 mM tricine/2 mM $MgCl_2$/30 mM NaCl, pH 7.5), using a Waring blendor working at full speed. The suspension was squeezed through a nylon tissue (mesh size, 24 μm), and then submitted to centrifugation (5 min at $2000 \times g$ at 5°C). Pellets were kept on ice, in the dark, for at least 3 h before the experiments. They were homogenized in darkness in a medium similar to the isolation medium, but with 10 mM KCl replacing NaCl, and then incubated in the dark for 30 min. In some experiments 50 μM potassium ferricyanide was added during the 30 min incubation, and then removed by one centrifugation before the experiment. In that case the pellet of chloroplasts was then rehomogenized in the same medium, without ferricyanide. In many experiments, when indicated, 5 mM hydroxylamine and/or 1 μM valinomycine were added to the chloroplasts and further incubated for 5 min in the dark.

Herbicides

Stock solutions of herbicides in ethanol were at 5–20 mM. Simazine and norazine were at 5 mM in acetone. Several herbicides were kindly supplied as gifts: INT, ioxynil and DNP-INT (Professor Trebst, Bochum), *i*-dinoseb and atrazine (Dr. Van Assche, Procida, Marseille), simazine and fluometuron (Dr. Hascoet and Mr. Jamet, INRA, Versailles), norazine (Ciba-Geigy). DNOC was a Fluka product. CMU and DCMU (K and K) were recrystallized twice from isopropanol.

Measurement of electron-transfer inhibition

Rates of DCIP photoreduction were measured as in Ref. 17, with a chlorophyll concentration of $1.5 \cdot 10^{-5}$ M. Herbicides were used in the 30–80% inhibition range to determine their I_{50} as in Ref. 18.

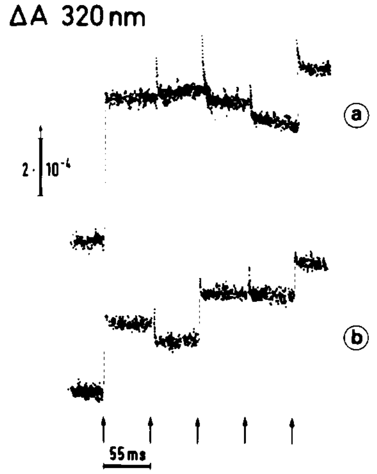
Measurement of time-resolved absorption changes

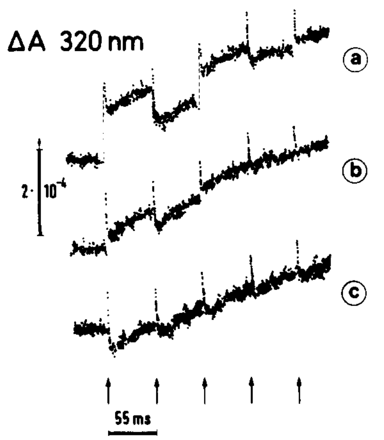
The method is that of Mathis and Haveman [8], with only minor modifications [19]. 200 ml of a suspension of chloroplasts, at a chlorophyll concentration of 15–20 $\mu g \cdot ml^{-1}$, were kept in darkness in a stirred reservoir thermostated at 9°C. By mean of a magnetic valve, the suspension was allowed to enter a 3.5 ml cuvette (optical path, 10 mm) about 1 s before being excited by a train of 5–10 saturating xenon flashes. After the train of flashes, a new sample was introduced by a 6.5 ml flush of dark-adapted suspension. The content of one reservoir thus served for 30 measurements, and was then either discarded or recycled one, two or three times, as indicated. The time interval between series of 30 measurements was 5 min. In some experiments (Table I), the flowing system was turned off; the cuvette was submitted to successive trains of flashes separated by an adjustable time interval (6–60 s). The electric bandwidth of the measuring system was d.c. to 3 kHz (or 10 kHz when indicated).

Results

Absorption changes without herbicides

Spinach chloroplasts were dark-adapted in the presence of 50 μM ferricyanide. The oxidant was then discarded by centrifugation and the chloroplasts submitted to a train of five saturating flashes. The time course of ΔA at 320 nm is shown in Fig. 1a. It resembles results obtained earlier [8,19] and interpreted essentially in terms of a period-4 signal due to the oxygen-evolving complex and of a period-2 signal due to Q_A and Q_B. After incubation with hydroxylamine, which inhibits the oxygen-evolving complex [20], the pattern of ΔA is simplified (Fig. 1b): a period-2 behaviour is observable, probably superimposed on a positive ΔA after each flash. It is noticeable that a fast tran-





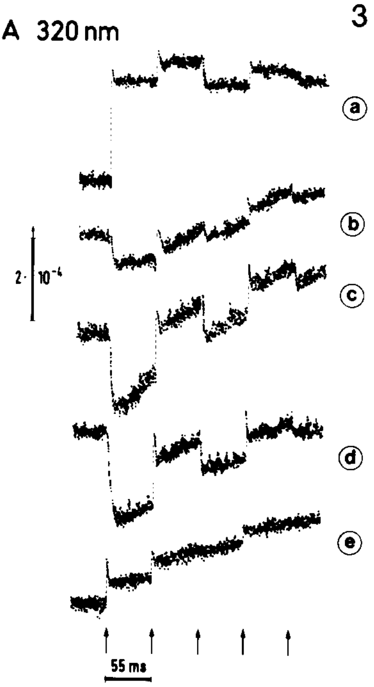


Fig. 1. Absorption changes induced at 320 nm in dark-adaptated spinach chloroplasts by a train of five flashes (50 μM ferricyanide was added during the dark adaptation and removed by centrifugation). Average of 60 measurements. Chlorophyll concentration: $2.5 \cdot 10^{-5}$ M. The arrows indicate the time at which flashes were fired. a, no addition; b, +5 mM hydroxylamine.

Fig. 2. Absorption changes induced at 320 nm by a train of flashes. Same conditions as Fig. 1, except: addition of 5 mM hydroxylamine and 1 μM valinomycine. a, first series of flashes; b, second series (after 5 min in darkness); c, fourth series.

Fig. 3. Absorption changes induced at 320 nm by a train of flashes, in spinach chloroplasts (chlorophyll concentration: 25×10^{-6} M for b, c, d, e, and $1.8 \cdot 10^{-5}$ M for a; average of 60 measurements), with 0.5 μm *i*-dinoseb. a, no further addition; b, addition of 5 mM hydroxylamine and 1 μM valinomycine, first series of flashes after dark adaptation; c, same as b, except fourth series of flashes; d, same sample as in c, with further addition of 1 μM dinitrophenyl-iodonitrothymol (DNP-INT); e, same as b, with chloroplasts previously dark-adapted with 50 μM ferricyanide (removed by centrifugation).

sient ($t_{1/2} \simeq 1$ ms) has a small size following the first, third and fifth flashes, whereas it is larger after the second and fourth flashes. This behaviour, which is predictable for the Q_A,Q_B couple [8], is not directly observable without hydroxylamine (Fig. 1a), presumably because of millisecond absorption transients from the oxygen-evolving complex [8,21].

The period-2 oscillation of the stable absorption level is better seen (Fig. 2a) after further addition of valinomycin/K^+, which was added in order to suppress an eventual contribution of electrochromic absorption shifts. It should be noted that, with this addition, the fast phases are due to both the Q_A,Q_B couple and the electrochromic shifts.

When the chloroplast suspension is recycled in the flowing system, and thus illuminated several times, the pattern of flash-induced ΔA evolves progressively: the period-2 transients are greatly reduced at the second passage, and completely absent at the third (Fig. 2). This evolution can be understood if the secondary quinone of PS II is largely oxidized (state Q_B) before the first series of flashes (whence the size and the sign of the period-2 oscillation at the first series), and that Q_B^- then accumulates. After further illumination (third series of flashes) it seems that the ratio Q_B/Q_B^- is about 1.

The traces of Fig. 2 also indicate a progressive and slow absorption increase within one train of five flashes (see also Fig. 5). This was observed only with hydroxylamine and is not understood for the moment.

Absorption changes in the presence of low concentration of i-dinoseb

When chloroplasts, with or without ferricyanide, were supplemented with 30 μM *i*-dinoseb, only the first flash induced at 320 nm a ΔA which was positive and stable, as in previous experiments with DCMU [8]. This indicates that Q_A is photoreduced, and stays reduced for at least 1 s. With a low concentration (0.5 μM) of *i*-dinoseb, without oxidant, a small period-2 ΔA is obtained, superimposed on a positive ΔA due to the first flash (Fig. 3a). It appears, however, that the sign of the period-2 ΔA is inverted compared to that of Fig. 2. When chloroplasts were dark-adapted and incubated in darkness with 5 mM hydroxylamine, practically only a period-2 ΔA of small amplitude was remaining when 0.5 μM *i*-dinoseb was added to a dark-adapted suspension (Fig. 3b), and about twice bigger when the inhibitor was added after a few cycles of flash preillumination (Fig. 3c). The pattern of flash-induced ΔA was hardly modified (Fig. 3d) by addition of 1 μM DNP-INT, an inhibitor of plastohydroquinone reoxidation by the plastoquinone-plastocyanine-oxidoreductase [12]. These results indicate that, under the conditions of Fig. 3c, a large fraction of the PS II centers are in the state $Q_AQ_B^-$ before the first flash. The pattern shown in Fig. 3c remained identical during many series of experiments, involving recycling of the chloroplasts in the flowing system. This behaviour is very different of what was observed in chloroplasts without herbicide (Fig. 2). The inverted

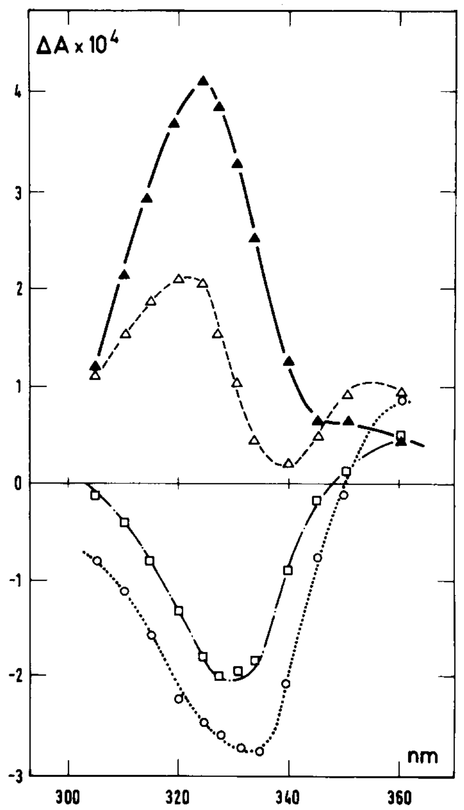


Fig. 4. Spectrum of absorption changes induced by the first (○), second (△) and third (□) flashes in a sequence, under conditions exactly identical to those of Fig. 3c. The spectrum in closed triangles (▲) is computed as 1·ΔA (second flash)−1.2·ΔA (third flash).

oscillations that we report here did not take place under oxidizing conditions (Fig. 3e; chloroplasts dark-adapted with ferricyanide).

The difference spectrum of ΔA induced under the conditions of Fig. 3c were measured for the first, second and third flashes, between 305 and 360 nm. As shown in Fig. 4, the maximum of ΔA is around 335 nm for the first flash, and near 325 nm for the second and third flashes. The spectrum of the species which displays a period-2 behaviour is better obtained by the difference between the ΔA due to the second and third flashes. This should permit to get rid of nonperiodic components and of an eventual first flash effect (see Ref. 11). This spectrum is shown in Fig. 4 (the third flash signal has been multiplied by 1.2 to correct from the damping between successive flashes). It has a well-defined peak at 325 nm which resembles that of a plastoquinone anion. After the first flash the spectrum is slightly different: peak at 335 nm, crossing point at 350 nm. We hypothesize that the first flash may include a contribution due to the oxidation of cytochrome *f*, since reduced cytochrome *f* has an absorption peak at 335 nm, and the oxidized species absorbs more at 360 nm [22]. Absorption measurements at 554 nm indicate that some cytochrome *f* is oxidized by the first flash, and much less by subsequent flashes as shown in Fig. 5. The same figure illustrates the damping of period-2 ΔA at 320 nm in a sequence of 10 flashes.

In the experiments of Fig. 3 submillisecond transients were not correctly obtained because of a bandwidth limitation at 3 kHz. An experiment with a 10 kHz bandwidth is shown in Fig. 6. Valinomycin was not added to avoid a possible disturbance by the rapidly relaxing electrochromic shifts. Fast transients can be resolved after the first and the third flashes, with a $t_{1/2}$ of 1.0 ± 0.2 ms. This corresponds to the time for electron transfer from Q_A^- to Q_B^- at 9°C, as reported earlier [8].

Effect of inhibitor nature and concentration

The behaviour depicted in Fig. 3 has been observed with inhibitors of Q_A^- reoxidation which belong to three different classes of herbicides: ureas, triazines, phenols [12]. For each of them we have studied the effect of concentration on flash-induced ΔA (first and second flash, as in Fig. 3c) and on steady-state electron transport in chloroplasts not treated with hydroxylamine. Some results of flash experiments are presented in Fig. 7, for *i*-dinoseb and DCMU. At low concentration, a true period-2 oscillation is observed, the size of which increases until a maximum. When the inhibitor concentration is raised above the maxi-

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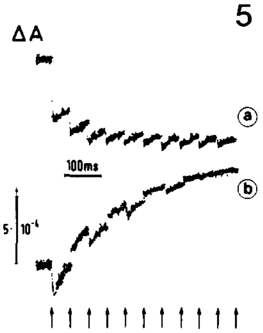

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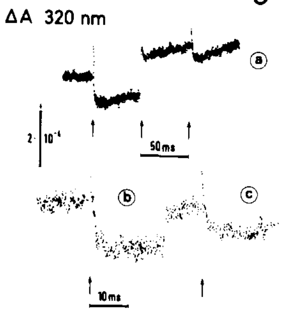

Fig. 5. Absorption changes at 554 nm (a) and 320 nm (b) induced by a train of ten flashes, under the conditions of Fig. 3c.

Fig. 6. Kinetics of absorption changes induced at 320 nm by the first three flashes in a sequence of five flashes, under the conditions of Fig. 3c, except that the electrical bandwidth is 10 kHz (100 μs per channel) and that no valinomycin was added. Bottom: enlarged view of ΔA at the first and third flashes. Average of 300 experiments.

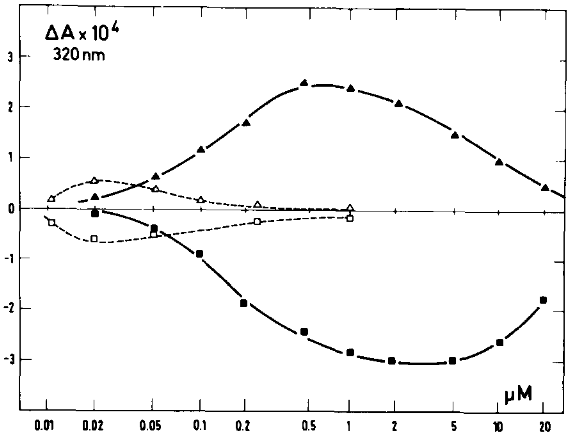

Fig. 7. Effect of inhibitor concentration (*i*-dinoseb, closed symbols; DCMU, open symbols) on ΔA, induced at 320 nm by the first (squares) and the second (triangles) flashes in a sequence of five flashes, with spinach chloroplasts under the conditions of Fig. 3c (except for inhibitor nature and concentration).

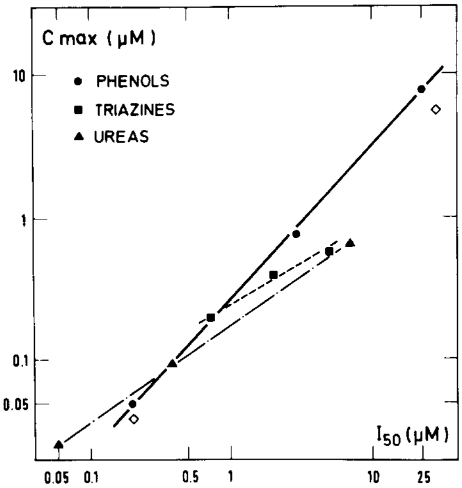

Fig. 8. Relation between I_{50} measured for different inhibitors and $C_{\max}$, the concentration which gives the maximum ΔA at 320 nm at the second flash. Urea-type inhibitors. ▲ (DCMU, CMU, fluometuron); triazines, ■ (atrazine, simazine, norazine); phenolic inhibitors, ● (INT, *i*-dinoseb, DNOC); others, ◇ (ioxynil, *o*l-phenanthroline). Within each group, the inhibitors are given in order of increasing I_{50}.

TABLE I

QUANTITATIVE PARAMETERS OBTAINED WITH SPINACH CHLOROPLASTS FOR SEVERAL INHIBITORS

I_{50} and $C_{\max}$ (in μM) are the concentrations of inhibitor which give, respectively, a 50% inhibition of the Hill reaction rate (see Methods) and maximum ΔA at the second flash (see Fig. 6). $GA_{\max}$ is a relative index of the ΔA at 320 nm, at the second flash, with a $C_{\max}$ concentration of inhibitor (conditions of Fig. 6). $t_{1/2}$ is the time separating successive flash group, which gives a half-maximum value of the ΔA at the second flash, in the presence of a $C_{\max}$ concentration of inhibitor.

	I_{50} (μM)	$C_{\max}$ (μM)	$\Delta A_{\max}$	$t_{1/2}$ (s)
Phenols				
DNOC	25	8	100	6
i-dinoseb	3	0.7	100	12
ioxynil	0.2	0.04	35	> 60
INT	0.2	0.05	35	> 60
Ureas				
Fluometuron	7	0.8	80	13
CMU	0.4	0.1	40	45
DCMU	0.06	0.025	25	> 60
Triazines				
Norazine	5	0.6	85	–
Simazine	2	0.4	70	–
Atrazine	0.7	0.2	45	25
Other				
o-phenanthroline	30	8	90	9

mum, the ΔA at the second flash decreases faster than the ΔA at the first flash; at the highest concentration used the oscillatory pattern is lost, since no ΔA is observed at the third and subsequent flashes. Three parameters have been measured in these experiments: I_{50} for steady-state electron transfer (see Material and Methods); $C_{\max}$, the inhibitor concentration which gives the maximum ΔA at the second flash; $\Delta A_{\max}$, the relative ΔA at the second flash, obtained for $C_{\max}$. As shown in Fig. 8, there are large variations among these parameters. Within each class of herbicide there is a good correlation between I_{50} and $C_{\max}$. $\Delta A_{\max}$ is smaller with the most active inhibitors, but its range of variation is only from 100 to 25 (Table I). The largest oscillations were obtained with the phenolic herbicides DNOC and *i*-dinoseb, the smallest with DCMU.

Recovery time between flash groups

For all the results reported above, the chloroplasts were kept for at least 5 min in darkness between two successive trains of flashes. The effect of this dark-adaptation time on the size of period-2 oscillations has been studied by turning off the flowing system and measuring the signal due to trains of five flashes separated by Δt. Under these conditions the amplitude of the oscillations (measured as the positive ΔA induced by the second flash) decreases at short Δt. The decrease is practically monotonous and a half-time of recovery ($t_{1/2}$) could be measured, as the time interval between flash groups for which the ΔA at the second flash attains 50% of its maximum value. As shown in Table I, this $t_{1/2}$ varies greatly, grossly in antiparallel with the I_{50} value.

Effect of herbicides on triazine-resistant chloroplasts

Chloroplasts from triazine-susceptible (wild type) *Chenopodium album* present the same pattern of flash-induced ΔA at 320 nm without herbicide or with a low concentration of atrazine or of *i*-dinoseb (Fig. 9A). Chloroplasts from a triazine-resistant biotype of chenopod give also the same results in the absence of herbicide (Fig. 9B), although the damping of oscillation (top trace) may be more rapid. Triazine has no effect, as expected (Fig. 9B). With *i*-dinoseb, however, no inverse oscillatory pattern could be detected in the

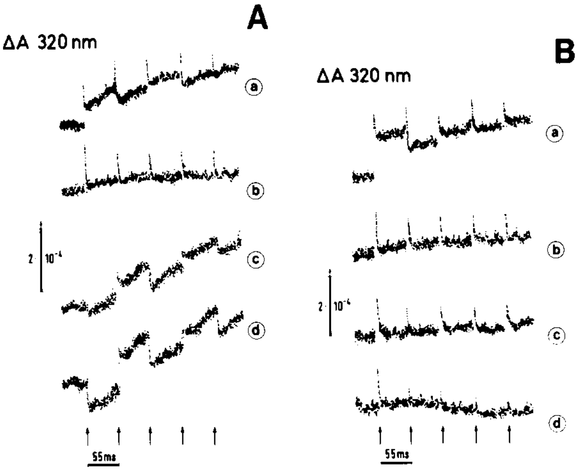

Fig. 9. Absorption changes at 320 nm induced in chloroplasts of *Chenopodium album* (chlorophyll concentration, 25 μM). A, atrazine-sensitive (wild-type) biotype; B, atrazine-resistant biotype. Addition of 5 mM hydroxylamine and 1 μM valinomycine. a, No further addition, first series of flashes after dark-adaptation; b, same as a, but fourth series of flashes; c, d, same conditions as b, with 0.8 μM atrazine or 0.5 μM *i*-dinoseb, respectively.

concentration range 50 nM–2 μM. The herbicide inhibits electron transfer, since only the first flash elicits a large signal (Fig. 9B, bottom). DCMU has the same effect as *i*-dinoseb (data not shown).

In Fig. 9A and B, top traces, it may be noticed that the size of the fast kinetic phases, due to the electron transfer from Q_A^- to Q_B^-, presents a period-2 oscillation, as in spinach chloroplasts.

In atrazine-susceptible chloroplasts the $t_{1/2}$ of fast transients is 0.9 ms, as in spinach chloroplasts, in the absence of valinomycine. In atrazine-resistant chloroplasts the $t_{1/2}$ is 1.4 ms (not shown). The slowing down of Q_A^- reoxidation in atrazine-resistant biotypes of several plant species had already been observed by fluorescence measurements [23,24].

Discussion

Flash-induced absorption changes in the near ultraviolet, with chloroplasts, are highly complex, as illustrated by Fig. 1a. Contributions of the electron donors to PS II are suppressed by addition of hydroxylamine: the signals of Fig. 1b are probably due mostly to the electron acceptors. A period-2 behaviour with flash number is clearly observable. It seems, however, to be superimposed on a positive ΔA (see flash number 4), the origin of which we do not know. Addition of valinomycin/K$^+$ permits to obtain simpler features (Fig. 2), with a clear period-2 pattern due to the Q_A,Q_B system. As discussed earlier [8,10], the results of Fig. 2a indicate that Q_B is oxidized before the first flash, by virtue of the oxidizing pretreatment with ferricyanide. As also shown in Fig. 2, preillumination of the chloroplasts induces a disappearance of the period-2 oscillations: this is attributed to the progressive accumulation of reaction centers in the state Q_B^-. In no case did we observe oscillations of inverted sign under the conditions of Fig. 2. This shows that no more that 50% of the centers can be in the state Q_B^- (addition of reductants does not change this conclusion, data not shown).

Inverse oscillations were observed only when a low concentration of herbicide was added and, additionally, only after a preillumination of the chloroplasts (Fig. 3). Several arguments permit to associate these inverse oscillations with the Q_B site: kinetics of transitory ΔA (Fig. 5), spectrum of the stable ΔA (Fig. 4), absence of effect of DNP-INT, disappearance of the oscillations with saturating inhibitor concentrations. The results of Fig. 3a, and c indicate that, before the first flash, there are more centers in the state Q_A,Q_B^- than in the state Q_A,Q_B (in addition, some centers are probably blocked in the state Q_A^- which is very stable in the presence of inhibitor and hydroxylamine). This situation has not been reported. Under optimum conditions we find that the excess of Q_B^- over Q_B correspond to 35% of the PS II centers. In view of other results concerning the functioning of Q_B (see Introduction), our observations can be considered as further proof that PS II centers in the state Q_B can bind a molecule of herbicide I (after one flash these centers will be blocked in the state Q_A^-I) and that PS II centers in the state Q_B^- do not bind the inhibitor, and give rise to the inverted period-2 oscillations.

As shown in Fig. 8 and Table I, there is a good correlation between the reported effects and the capacity of various herbicides to inhibit steady-state electron transfer. The influence of concentration is easily understandable: there must be enough inhibitor to bind to some centers in state Q_B, but not too much to avoid blocking many centers in state $Q_A^- I$ or to bind to Q_B centers in the interval between two flashes. Within a train of flashes the amplitude of stable ΔA induced by each flash decreases progressively (Fig. 3 and 5). This may be due to the progressive accumulation of centers in the state Q_A^- and to a dephasing in the other centers (because of double hits, misses, inhibitor unbinding, etc.). The time required to recover a full-size oscillation between two trains of flashes varies between inhibitors (Table I). It corresponds presumably to the evolution of centers $Q_A^- I$ into $Q_A Q_B^-$ (inhibitor unbinding) and of centers $Q_A Q_B$ into $Q_A I$ (inhibitor binding). The recovery time is thus an indication of the rate of inhibitor reactions at the Q_B site. Our results can be compared in this respect with those of Vermaas et al. [25], who concluded to fast rates of binding and unbinding for *i*-dinoseb, dinitro-*o*-cresol and *o*-phenanthroline, and to slow rates for DCMU and atrazine. This fits nicely with our recovery time measurements (Table I). Vermaas et al. [25] concluded also that the inhibitor/quinone exchange mainly takes place in state Q_B^- in the case of *i*-dinoseb. This proposal is not supported by our observations. It is quite possible that the extra damping of flash-induced oxygen evolution observed by Vermaas et al. with *i*-dinoseb is due to extra misses at the donor side due to the carotenoid oxidation appearing with phenolic herbicides [26]. As shown in Table I, the recovery time (and thus presumably the time of inhibitor binding/unbinding) varies antiparallel with the inhibitor efficiency.

The large variation in the maximum amplitude of oscillations between different inhibitors (Table I) might be related to differences in turn-over times. However, with a possible exception for DCMU, the dark-adaptation time between flash sequences in the measurement of $\Delta A_{\max}$ was well above the recovery time.

Chloroplasts from wild-type chenopods exhibit the same period-2 ΔA as spinach chloroplasts; low concentrations of *i*-dinoseb and atrazine lead to inverse oscillations (Fig. 9A). In the atrazine-resistant biotype, the normal pattern is observed in the absence of herbicide (Fig. 9B). The more rapid damping may be due to an incomplete electron transfer from Q_A^- to Q_B (or Q_B^-) between flashes, in agreement with previous fluorescence kinetic observations [23,24]. We also found that the $t_{1/2}$ of the fast phase of electron transfer is longer in the mutant than in the wild type (1.4 ms instead of 0.9 ms), as already reported in several plant species [23,24]. The most surprising property of atrazine-resistant chloroplasts is that inverse oscillations could not be obtained in the presence of *i*-dinoseb (compare Fig. 9A and B) or DCMU, even at very low concentrations. This might indicate that Q_B an Q_B^- are nearly equally bound at their site, in line with the proposal by Bowes et al. [24] that Q_B is more firmly bound to its apoprotein in atrazine-resistant than in wild-type *Amaranthus* chloroplasts. However, as shown in Fig. 9A and B (traces a and b), the atrazine-susceptible and the atrazine-resistant chloroplasts display nearly identical ΔA patterns in the absence of herbicide, showing that the Q_B/Q_B^- equilibrium seems to be the same in both biotypes. The absence of inverse oscillations with *i*-dinoseb and DCMU in atrazine-resistant chloroplasts might indicate a tighter binding of herbicides to the Q_B sites in the mutant, as suggested by others [27–29].

Acknowledgements

We thank Drs. Hascoet, Jamet, A. Trebst and C.J. Van Assche for kindly providing herbicides, and Drs. Ducruet and El Anouar for providing the chenopod seeds. We have also benefited from useful discussions with Dr. A.W. Rutherford.

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