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FURTHER RESULTS ON THE PHOTOACTIVE CHLOROPHYLL a_{II} IN PHOTOSYNTHESIS

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SUMMARY

1. The flash-induced difference spectra of chlorophyll a_{II} in untreated and in heat-treated spinach chloroplasts are presented.

2. Heat-treatment leads to an increase of the half-life of the chlorophyll a_{II} absorbance changes. After the addition of hydroquinone plus sodium ascorbate the half-life of the chlorophyll a_{II} reaction returns nearly to the value in untreated chloroplasts.

3. In digitonin-treated chloroplasts, or in the presence of histone, the chlorophyll a_{II} activity is low when the electron acceptor is benzylviologen and high when ferricyanide is used.

4. The half-life of the chlorophyll a_{II} reaction depends on the temperature of the reaction mixture. The activation energy of the chlorophyll a_{II} reaction is about 7.5 kcal/mol.

5. From these experimental results we conclude that in heat-treated (and in Tris-washed and aged) chloroplasts the linear electron flow in Photosystem II is replaced by a cyclic one, which is sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

INTRODUCTION

The electron transport of photosynthesis from water to $NADP^+$ is driven by two light reactions in series [1–3]. The active pigments are chlorophyll a_I [1, 4] and chlorophyll a_{II} [5, 6]. They are linked by several intermediates including the plastoquinone pool PQ and the primary electron donors PD of chlorophyll a_I (see Fig. 1).

It is well established that chlorophyll a_I is engaged in the electron transport chain with a redox reaction [7, 8]. The decision whether the type of reaction is a redox reaction or a sensitizer reaction is much more difficult for chlorophyll a_{II}.

Tris washing, heating and aging have the same effect on the chloroplasts: Mn^{2+} is released in parallel with inactivation [9, 10], and the electron transport chain

Abbreviations used: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium sulphate.

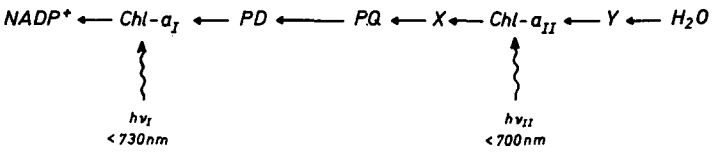

Fig. 1. Simplified scheme of the electron transport chain (25). PD includes cytochrome *f* and plastocyanin. Reduced X has been identified as a special plastosemiquinone anion [16, 17]. Y is an unknown carrier. Chl, chlorophyll; PQ, plastoquinone.

is interrupted between the water-splitting site and chlorophyll a_{II}. In such chloroplasts there is no electron transport from water to viologen, but the chlorophyll a_{II} reaction is still active [6, 11]. These results can be interpreted in two ways:

(1) Chlorophyll a_{II} is not engaged directly in the electron transport chain but acts as a sensitizer, which is still active when there is no electron transfer from Y to X.

(2) Chlorophyll a_{II} is engaged directly in the electron transport chain with a redox reaction or with a sensitizer reaction which is coupled with an electron transfer from Y to X. In Tris-washed, in heat-treated and in aged chloroplasts a cyclic electron flow around chlorophyll a_{II} takes place.

In refs 6 and 11 the second possibility was assumed to be improbable because the half-life of the chlorophyll a_{II} absorbance changes in Tris-washed, in heat-treated and in aged chloroplasts was nearly the same as in untreated chloroplasts, and because in the presence of artificial electron donor systems for Photosystem II there is a linear electron flow from the donor to $NADP^+$ [16]. Therefore, it was assumed that chlorophyll a_{II} acts as a sensitizer which is not coupled with an electron transfer from Y to X.

In Tris-washed, in heat-treated and in aged chloroplasts the chlorophyll a_{II} reaction is sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Based on the assumption that chlorophyll a_{II} acts as a sensitizer which is not coupled with an electron transfer from Y to X it was concluded from these results that the reaction site of DCMU must be chlorophyll a_{II} [6]. But this is in contradiction to the results of Malkin and Kok [13], Forbush and Kok [14] and Duysens and Sweers [15] and others. Adopting the assumption that the fluorescence yield is associated with the redox state of the primary electron acceptor of Photosystems II (X as shown by Witt, K. [16]*), they concluded, from measurements of fluorescence induction curves, that the reaction site of DCMU must be between this primary electron acceptor of Photosystem II and a pool of secondary oxidant (the plastoquinone pool PQ in the scheme of Stiehl and Witt, H. T. [17]). Although recent results of Mauzerall [18] have brought the primary nature of fluorescence yield changes into question, new results of Witt, K. [16] suggest that DCMU blocks the electron transport between X and PQ.

The results of our investigations of the chlorophyll a_{II} absorbance changes are compatible with the results of Kok, Duysens and Witt, K. if we assume that the chlorophyll a_{II} reaction is either a redox reaction or a sensitizer reaction which is coupled with an electron transfer from Y to X. The first assumption seems to be more probable (see Discussion).

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* The bandshift C 550 has also been proposed to be associated with the primary acceptor of Photosystem II [19, 20].

MATERIALS AND METHODS

Stripped spinach chloroplasts have been isolated as described in [21]. Suspensions enriched with Photosystem II have been prepared according to Anderson and Boardman [22]. The spectroscopic measurements were performed by the repetitive flash technique described in [5]. Excitation: 385–500 nm (2 mm BG 28+2 mm KG 2 from Schott), 610–710 nm (4 mm RG 610+2 mm KG 2 from Schott), saturating flashes of 20 μs duration. The electrical band width ranged from 0.1 Hz to 30 kHz. The optical path length through the cuvette was 1.2 mm. The band width of the monitoring light (grating monochromator) was 5 nm, the intensity about 50 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. 10^3 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} $h\nu_I$ background light (716 nm) were irradiated. The temperature of the sample was 22 °C.

The chlorophyll concentration was 10^{-4} M. The sample contained 0.05 M Tris/HCl buffer (pH 7.2), 10^{-4} M benzylviologen respectively $5 \cdot 10^{-4}$ M ferricyanide as electron acceptor, and NH_4Cl $2 \cdot 10^{-3}$ M as phosphorylation uncoupler. For the measurements with histone all salts have been washed away from the chloroplasts before histone was added. Further details are given in the legends of the figures. Deviating conditions are noted.

RESULTS

Difference spectra of chlorophyll a_{II}

The difference spectrum of chlorophyll a_{II} published in 1969 [6] was not measured in whole chloroplasts, but in subchloroplast particles (10 000$\times g$ fraction of digitonin-treated chloroplasts). For a long time we could not find the Soret band of chlorophyll a_{II} in untreated chloroplasts because absorbance changes of cytochrome *f* were superimposed. Now this difficulty has been ruled out by irradiation with strong $h\nu_I$ background light ($5 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} (720 nm)). In the dark time between the exciting flashes cytochrome *f* is held in the oxidized state by the $h\nu_I$ light and shows now absorbance changes during and after the exciting flashes. Under these conditions the difference spectrum shown in Fig. 2, top has been measured. The absorbance changes have a half-life of about 150 μs and are maximal at 433 and 687 nm.

The chlorophyll a_{II} reaction can be separated from the overall electron transport by heating the chloroplasts for 5 min at 50–55 °C [6]. The flash-induced difference spectrum of chlorophyll a_{II} in heat-treated chloroplasts (5 min at 53 °C) is presented in Fig. 2 (bottom). The absorbance changes have a half-life of about 250 μs and are maximal at 437 and 683 nm. As seen in Fig. 2, the difference spectra of chlorophyll a_{II} in heat-treated and in untreated chloroplasts differ only slightly from that in digitonin-treated chloroplasts (see [6]). The absorbance changes are maximal in the regions of 435 and 685 nm in all these cases.

It should be noted that the half-life of the chlorophyll a_{II} absorbance changes is not always about 150 μs in untreated and 250 μs in heat-treated chloroplasts, but that these half-lives change from preparation to preparation.

The sensitivity of the chlorophyll a_{II} absorbance changes to DCMU has been studied in the three types of chloroplasts at 435 nm and at 685 nm. Heat-treatment and digitonin-treatment do not alter the sensitivity of the chlorophyll a_{II} absorbance changes to DCMU: in all these cases the chlorophyll a_{II} absorbance changes depend

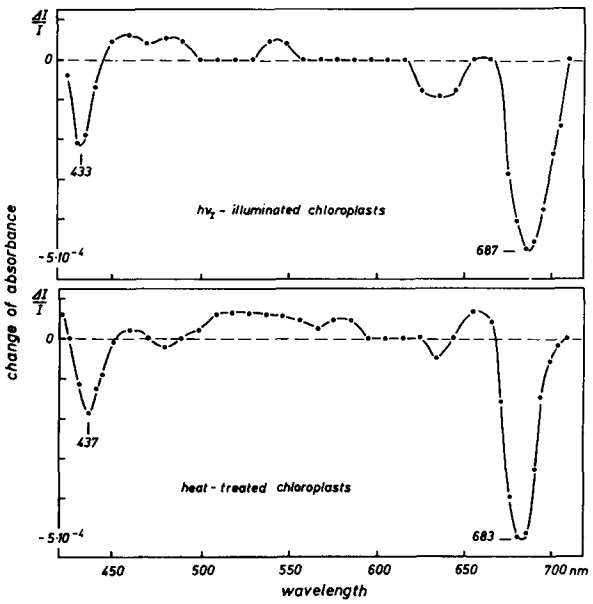

Fig. 2. Top. Absorbance changes with a life time of approx. 150 μs as a function of the wavelength in stripped spinach chloroplasts with benzylviologen as electron acceptor. Activity of O_2 production: 151 moles O_2/(mole chlorophyll per h). Repetition rate 8 Hz. Intensity of the $h\nu_I$-background light $5 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} (720 nm). Bottom. Absorbance changes with a life time of approx. 250 μs as a function of the wavelength in heated spinach chloroplasts (5 min at 53 °C) with benzylviologen as electron acceptor. No activity of O_2 production. Repetition rate 8 Hz.

in the same way on the DCMU concentration as the relative O_2 yield per flash in untreated chloroplasts. The concentration for half inactivation is 0.1–0.2 μM.

Measurements in chloroplasts, in which the electron transport is damaged in Photosystem II

Heat-treated chloroplasts. The amplitude of the chlorophyll a_{II} absorbance changes at 685 nm is resistant to a 5-min heat treatment up to 50 °C. The temperature of half inactivation (in 5 min) is 55–56 °C. This was reported already in [6]. In contrast to the amplitude, the half-life of the chlorophyll a_{II} absorbance changes depends on the temperature of heat-treatment even at temperatures lower than 50 °C. As shown in Fig. 3 the half-life rises from about 120 μs in untreated chloroplasts up to about 250 μs in chloroplasts pretreated at 54 °C.

The chlorophyll a_{II} absorbance changes following heat-treatment are diminished by $h\nu_{II}$ background light but not by $h\nu_I$ background light.

The addition of ascorbate plus benzohydroquinone, which is an artificial electron donor system for Photosystem II, to heat-treated chloroplasts (5 min at 52 °C) makes the kinetics of the chlorophyll a_{II} absorbance changes get nearly as fast as before heat-treatment, but the amplitude is diminished to 60 % of the value in untreated chloroplasts. The amplitude of the chlorophyll a_I absorbance change, which is diminished nearly completely by heat-treatment, is restored to 60 % after the addition of ascorbate plus benzohydroquinone to heat-treated chloroplasts.

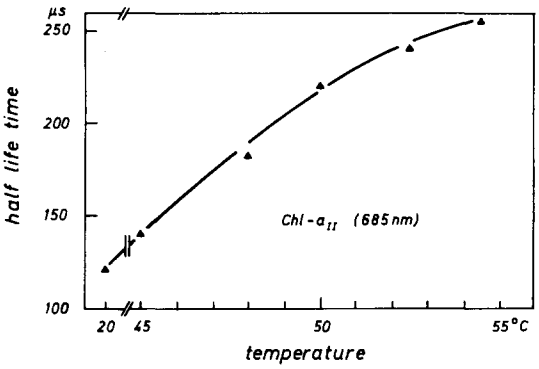

Fig. 3. Half-life of chlorophyll a_{II} (Chl-a_{II}) as a function of the heating temperature in stripped spinach chloroplasts with benzyl viologen as electron acceptor. The chloroplasts had been exposed to the indicated temperatures for 5 min before the measurement. During the measurement the temperature in the sample cuvette was 22 °C. Activity of O_2 production (before heating) 108 moles O_2/(mole chlorophyll per h). Repetition rate 8 Hz.

In the presence of 2 μM DCMU or after heat treatment at 60 °C (5 min) the addition of ascorbate plus hydroquinone cannot reactivate the electron transport via chlorophyll a_{II} and chlorophyll a_I to benzylviologen.

Measurements in chloroplasts, in which the electron transport is damaged in photosystem I

Digitonin-treated chloroplasts. The absorbance changes of chlorophyll a_I (705 nm) and chlorophyll a_{II} (690 nm) and the ability to reduce ferricyanide have been investigated in the $10\,000 \times g$ fraction of digitonin-treated chloroplasts, see Materials and Methods, with different additions.

In these subchloroplast particles the electron transport chains are interrupted between the two photosystems, because most of the cytochrome *f* [25] and plastocyanin [26, 27] is removed. If benzylviologen is used as electron acceptor and no artificial electron donor is present, both the chlorophyll a_I and the chlorophyll a_{II} activities are low (10–20 % of the activity in untreated chloroplasts), indicating that in our subchloroplast particles only 10–20 % of the electron transport chains are intact. With ascorbate plus *N*-methylphenazonium sulphate (PMS) as an artificial electron donor system and benzylviologen as electron acceptor the subchloroplast particles show full System I activity, but no System II activity (no chlorophyll a_{II} absorbance changes). If the electron acceptor is ferricyanide and no artificial electron donor is used, they show mainly System II activity (70–80 % chlorophyll a_{II} activity, 70–80 % Hill activity, 20–30 % chlorophyll a_I activity).

Histone-treated chloroplasts. From measurements of the $NADP^+$ reduction and the ferricyanide reduction Krogmann et al. [28] concluded that histone inhibits the electron transport in Photosystem I with greater efficiency than in Photosystem II.

The influence of histone on the two photosystems has been investigated by measurements of the absorbance changes of chlorophyll a_I (705 nm) and chlorophyll a_{II} (685 nm). As shown in Fig. 4, for 50 % inhibition of chlorophyll a_{II} about the 10-fold histone concentration is necessary as for chlorophyll a_I, when ferricyanide is

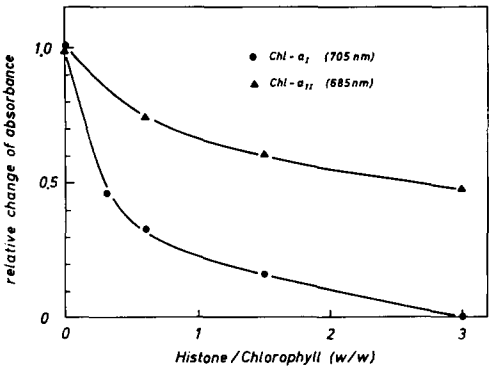

Fig. 4. Relative change of absorbance of chlorophyll a_I and chlorophyll a_{II} as a function of the ratio histone/chlorophyll (w/w) in salt free stripped spinach chloroplasts. Electron acceptor ferricyanide. Activity of O_2 production (without histone) 97 moles O_2/(mole chlorophyll per h). Repetition rate 3 Hz.

used as electron acceptor. These measurements were carried out with a repetition rate of 3 Hz. At higher repetition rates the inhibitory effect of histone is slightly less. These results are in good agreement with the findings of Renger [29] that histone does not influence the O_2 production even at concentrations which inhibit the chlorophyll a_I reaction completely.

Benzylviologen is an electron acceptor for Photosystem I [30], ferricyanide for Photosystem I [30] and for Photosystem II [31, 32]. Therefore, it is to be expected that in the presence of histone the activities of both chlorophyll a_I and chlorophyll a_{II} are low when the electron acceptor is benzylviologen, and that the activity of chlorophyll a_I is low and that of chlorophyll a_{II} is high when the electron acceptor is ferricyanide. These expections are verified by the results shown in Table I.

It should be noted that in the presence of viologen the chlorophyll a_I reaction can be reactivated by the addition of the artificial electron donor system ascorbate plus PMS. Therefore, the reaction site of histone cannot be the chlorophyll a_I molecule itself. This is in agreement with the results of Krogmann and co-workers [33, 34].

TABLE I

THE INHIBITORY EFFECT OF HISTONE ON THE ABSORBANCE CHANGES OF CHLOROPHYLL a_I AND CHLOROPHYLL a_{II}

The inhibitory effect of histone on the absorbance changes of chlorophyll a_I and chlorophyll a_{II} in the presence of benzylviologen and ferricyanide as electron acceptor in salt free spinach chloroplasts. Histone/chlorophyll (w/w) = 3 : 1. Repetition rate 8 Hz.

	Chlorophyll a_I (705 nm)		Chlorophyll a_{II} (685 nm)	
Electron acceptor	viologen	ferricyanide	viologen	ferricyanide
Control	100 %	100 %	100 %	100 %
+0.3 mg histone	5 %	8 %	7 %	55 %

The activation energy of the chlorophyll a_{II} reaction

The half-life of the chlorophyll a_{II} absorbance changes depends on the temperature in the sample cuvette. At room temperature (22 °C) the half-life is approx. 110 μs, but with decreasing temperature the chlorophyll a_{II} reaction gets slower, and at 2 °C the half-life is approx. 265 μs. From plotting the logarithm of the rate constant, k, as a function of the reciprocal absolute temperature (Fig. 5) the activation energy of the chlorophyll a_{II} back reaction in the dark is estimated to be approx. 7.5 kcal/mol.

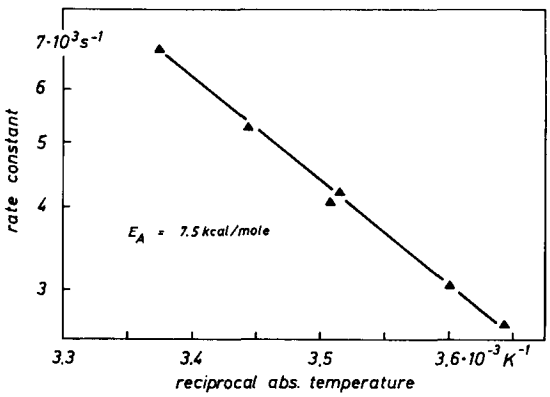

Fig. 5. Logarithm of the rate constant of the chlorophyll a_{II} reaction as a function of the reciprocal absolute temperature in stripped spinach chloroplasts. Electron acceptor benzylviologen. Activity of O_2 production at room temperature 110 moles O_2/(mole chlorophyll per h). Repetition rate 8 Hz.

DISCUSSION

The experiments reported in this paper and in [6] and in [11] have shown:

(1) When the electron flow is blocked between the water splitting site and chlorophyll a_{II} (Tris, heat, age), the chlorophyll a_{II} reaction is active and as sensitive to DCMU as in untreated chloroplasts.

(2) When the electron flow is blocked between the two photosystems (digitonin, histone), the chlorophyll a_{II} reaction is active and as sensitive to DCMU as in untreated chloroplasts when ferricyanide is used as electron acceptor, but inactive when viologen is used as electron acceptor.

(3) The half-life of the chlorophyll a_{II} absorbance changes increases with increasing temperature of heat treatment. When ascorbate, plus hydroquinone is added to heat-treated chloroplasts, the chlorophyll a_{II} absorbance changes become nearly as fast as in untreated chloroplasts.

(4) The half-life of the chlorophyll a_{II} absorbance changes increases with decreasing temperature in the sample cuvette.

In order to answer the question, whether chlorophyll a_{II} acts in a sensitizer reaction or in a redox reaction, we have to define what we understand by "sensitizer reaction". Any reaction of chlorophyll a_{II} which leads to an electron transfer from Y to X without an electron donation or acceptance by chlorophyll a_{II} itself, we call a sensitizer reaction.

(1) *Exclusion of a sensitizer reaction which is active even without an electron transfer*

In the case of a sensitizer reaction the 200 μs of the chlorophyll a_{II} absorbance changes cannot be a time constant of the electron transport, because the chlorophyll a_{II} molecules themselves do not accept or donate electrons.

For sensitizer reactions as defined above we can distinguish two cases:

(1) The chlorophyll a_{II} reaction is active even without an electron transfer from Y to X,

(2) the chlorophyll a_{II} reaction is active only when an electron is transferred from Y to X.

The first case would be compatible with our findings that the chlorophyll a_{II} reaction is active in Tris-washed, in heat-treated and in aged chloroplasts. But our results in digitonin-treated and in histone-treated chloroplasts (high chlorophyll a_{II} activity with ferricyanide, low chlorophyll a_{II} activity with viologen as electron acceptor) exclude this possibility, because the chlorophyll a_{II} activity should be high, regardless of the electron acceptor.

Furthermore, a sensitizer reaction which does not need an electron transfer from Y to X can be ruled out since Kok et al. [13, 14], Duysens et al. [15] and recently Witt, K. [16] have shown that the reaction site of DCMU must be between the primary electron acceptor of chlorophyll a_{II} (X) and the plastoquinone pool (PQ). In the case where the chlorophyll a_{II} reaction does not need an electron transfer from Y to X, either the chlorophyll a_{II} reaction should be insensitive to DCMU (ruled out by our experimental results), or DCMU should have two reaction sites with the same efficiency (one between X and PQ and one at chlorophyll a_{II}), which can be ruled out as very improbable*.

(2) *Exclusion of sensitizer models which are coupled with an electron transfer*

Such a sensitizer will be hard to distinguish from a redox reaction by experimental results. The experimental results reported in this paper and in [6] and in [11] can be interpreted if the chlorophyll a_{II} reaction is a redox reaction as well as if it is a sensitizer reaction which is coupled with an electron transfer from Y to X. An exception is the finding that the chlorophyll a_{II} absorbance changes become slower during heat treatment but faster again after the addition of ascorbate plus hydroquinone. Since in the sensitizer model the half-life of the chlorophyll a_{II} absorbance change is no measure of electron transfer, it is hard to understand how the above electron donor system accelerates the chlorophyll a_{II} reaction in heat-treated chloroplasts.

(3) *Model proposed for the reaction mechanism in Photosystem II*

In the case of either a sensitizer reaction or a redox reaction, the experimental results lead to the conclusion that in Tris-washed, in heat-treated and in aged chloroplasts the linear electron flow in Photosystem II is replaced by a cyclic one including X, chlorophyll a_{II} (in the case of a redox reaction) and Y. This cyclic electron flow must be sensitive to DCMU. The existence of a DCMU-sensitive cyclic electron flow

* Recently from a comparison of the relative O_2 yield per flash as a function of the time t_d between the exciting flashes in the absence and in the presence of 0.2 μM DCMU (this concentration has an inhibitory effect of 80 % for t_d = 100 ms) Renger [35] has concluded that DCMU acts also on the oxidizing side of Photosystem II. But under the conditions of our measurements (t_d = 125 ms) this effect can be neglected.

in Photosystem II in Tris-washed chloroplasts which does not exist in untreated chloroplasts has been postulated by Rosenberg et al. [36] (the reaction site of DCMU in this cyclic electron flow is assumed to be on the oxidizing side of chlorophyll a_{II}).

A proposed model for the mechanism of this cyclic electron flow must be compatible with the following experimental results:

(1) The cyclic electron flow in photosystem II is present in Tris-washed, in heat-treated and in aged chloroplasts, has the same sensitivity to DCMU as the linear electron flow in untreated chloroplasts, and is not present in digitonin-treated and in histone-treated chloroplasts.

(2) The chlorophyll a_{II} reaction becomes slower during heat-treatment, but becomes faster again after the addition of the electron donor system for Photosystem II ascorbate plus hydroquinone.

Because of difficulty in explaining the results set out under item 2 with the sensitizer model, we tend to the assumption that the chlorophyll a_{II} reaction is a redox reaction rather than a sensitizer reaction. For the reaction mechanism in Photosystem II we propose the following model.

In untreated chloroplasts the reaction in Photosystem II may have this mechanism:

$$XChlY_1Y_2 \xrightarrow{h\nu} XChl^*Y_1Y_2 \xrightarrow{<20\,ns} X^-Chl^+Y_1Y_2 \xrightarrow{200\,\mu s} X^-ChlY_1{}^+Y_2 \longrightarrow$$
$$\longrightarrow X^-ChlY_1Y_2{}^+ \longrightarrow X^-ChlY_1Y_2 \longrightarrow XChlY_1Y_2.$$

The reaction from $X^-ChlY_1{}^+Y_2$ to $XChlY_1Y_2$ needs 600 μs [37]. Y_1 and Y_2 are the direct electron donors of chlorophyll a_{II}.

When the electron flow is blocked between the water splitting site and Y_2 by Tris washing, heat-treatment or aging, in the first flash the mechanism is running until we have $X^-ChlY_1Y_2{}^+$. X^- donates the electron to PQ; the rereduction of $Y_2{}^+$ is prevented because the water splitting cannot donate electrons. Therefore, just before the second flash is fired, Photosystem II is in the state $XChlY_1Y_2{}^+$ (in untreated chloroplasts it was $XChlY_1Y_2$). By analogy the second flash leads to the state $XChlY_1{}^+Y_2{}^+$ and the third flash to $XChl^+Y_1{}^+Y_2{}^+$. The existence of the three positive charges makes the electron of X^- go to $Y_2{}^+$ rather than to PQ, so that in 200 μs Photosystem II relaxes from $X^-Chl^+Y_1{}^+Y_2{}^+$ to $XChlY_1{}^+Y_2{}^+$:

$$X^-Chl^+Y_1{}^+Y_2{}^+ \xrightarrow{<20\,\mu s} XChl^+Y_1{}^+Y_2 \xrightarrow{k_2} XChl^+Y_1Y_2{}^+ \xrightarrow{k_1} XChlY_1{}^+Y_2{}^+.$$

All further flashes induce the same reaction as the third one. The electron transfer from X^- to $Y_2{}^+$ must be sensitive to DCMU, and it must be faster than 20 μs (this is the time resolution of our equipment), because we do not detect absorbance changes of X in Tris-washed, in heat-treated and in aged chloroplasts.

In this model the condition for a cyclic electron flow in Photosystem II is that Photosystem I is in the state $XChlY_1{}^+Y_2{}^+$ when an exciting flash is fired. This condition is not complied with in (a) untreated, digitonin-treated and histone-treated chloroplasts, because $Y_1{}^+$ and $Y_2{}^+$ become reduced by the water splitting, and (b) Tris-washed, heat-treated and aged chloroplasts, when an artificial electron donor for Photosystem II is present, because $Y_1{}^+$ becomes reduced by this donor (see below).

Therefore, in these cases a cyclic electron flow in Photosystem II is not pos-

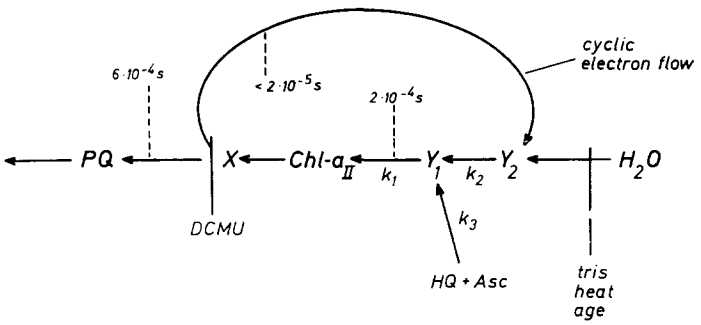

Fig. 6. Simplified scheme of the electron transport in heat-treated, in aged and in Tris-washed spinach chloroplasts. The $6 \cdot 10^{-4}$ s for electron transfer from X to plastoquinone has been estimated by Stiehl [37]. For details see text. HQ, hydroquinone; Asc, ascorbate.

sible. In Tris-washed, in heat-treated and in aged chloroplasts without an artificial electron donor in repetitive measurements, Photosystem II is in the state $XChlY_t^+Y_2^+$ when an exciting flash is fired, therefore a cyclic electron flow in Photosystem II is possible.

The half-life of the chlorophyll a_{II} absorbance changes depends on the rate constants k_1, k_2 and k_3 (see Fig. 6). In untreated chloroplasts, $k_2 \gg k_1$, and k_1 has a half-life of approx. 150 μs. During heat treatment k_1 does not change, but k_2 becomes smaller and makes the half-life of chlorophyll a_{II} increase. After the addition of ascorbate plus hydroquinone to heat-treated chloroplasts, $k_3 \gg k_2, k_1$, therefore the reduction of chlorophyll a_{II} becomes faster again.

It should be noted that the reaction $S_1^* \rightarrow S_2$ in the four step-model of Kok et al. [38] of the O_2 evolution has a first half-life $\leqslant 200$ μs at room temperature, similar to the half-life of chlorophyll a_{II}. Bouges [39, 40] has shown that the half-life of S_1^* depends on the temperature in the reaction vessel. Even though the temperature coefficient of the reaction $S_1^* \rightarrow S_2$ is slightly different from that of the chlorophyll a_{II} reaction, one cannot exclude that the reduction of chlorophyll a_{II}^+ after flash excitation is limiting for $S_1^* \rightarrow S_2$.

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