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THE EXISTENCE OF A HIGH PHOTOCHEMICAL TURNOVER RATE AT THE REACTION CENTERS OF SYSTEM II IN TRIS-WASHED CHLOROPLASTS

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Summary

In Tris-washed chloroplasts the kinetics of the primary electron acceptor X 320 of reaction center II has been investigated by fast repetitive flash spectroscopy with a time resolution of ≈ 1 μs. It has been found that X 320 is reduced by a flash in $\leqslant 1$ μs. The subsequent reoxidation in the dark occurs mainly by a reaction with a 100—200 μs kinetics. The light-induced difference spectrum confirms X 320 to be the reactive species. From these results it is concluded that in Tris-washed chloroplasts the reaction centers of System II are characterized by a high photochemical turnover rate mediated either via rapid direct charge recombination or via fast cyclic electron flow.

The linear System II electron flow from water to the plastoquinone pool mediated by the photochemically active reaction center C_{II} can be interrupted by 3 different types of inhibitions (for reviews see refs. 1,2): (a) DCMU[3(3,4-dichlorophenyl-1,1-dimethylurea)]-type blockage of electron efflux from C_{II} into the plastoquinone pool or (b) Tris-washing-type blockage of electron influx into C_{II} from water or (c) direct destruction of C_{II}. In Tris-washed chloroplasts, the linear System II electron transport can be easily restored by artificial electron donors (instead of water) indicating that the water-splitting enzyme system Y is selectively inhibited by this procedure [3,4]. Hence, the Tris-washing type blockage has been widely used as a tool for the functional separation of System II electron transport from the water-splitting enzyme system Y. In the absence of artificial electron

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

†Deceased on July 22, 1975. I dedicate this paper to my friend Christoph Wolff.

donors, C_{II} should be functionally blocked due to electron depletion on its donor side. However, it was found that chlorophyll a_{II} known to be the primary electron donor of C_{II} [5] remains fully active in heat-treated [6] or Tris-washed chloroplasts [7] under conditions where oxygen evolution completely disappeared. Hence, it has been concluded that in these treated chloroplasts a cyclic electron flow occurs around C_{II} [8]. In intact reaction centers C_{II} the light-induced electron transfer takes place from a_{II} to the primary electron acceptor X 320 [9,10,11]. Hence, if C_{II} remains uneffected by Tris-washing or mild heat treatment the amplitude of the absorption changes due to the redox reactions of X320 should not be significantly impaired. However, Döring did not observe absorption changes of X 320 in treated chloroplasts. Therefore, he inferred that the reoxidation of reduced X 320^- probably became too fast to be detectable by the applied measuring device [8]. In order to clarify the redox kinetics of X 320 in treated chloroplasts, the absorption changes of X 320 have been measured in Tris-washed chloroplasts with an apparatus allowing a time resolution of 1 μs.

Normal and Tris-washed chloroplasts have been prepared as decribed in ref. 12. The absorption changes in the ultraviolet region were measured according to refs. 9 and 10. In order to improve the time resolution, chloroplasts were excited by an ultra-short flash lamp [13], 1024 signals were averaged in a FABRI-Tek, Mod. 1062. The electrical bandwidth was 0—300 kHz. The reaction mixture is given in the legends to the figures.

In Fig. 1 the absorption changes at 334 nm are depicted for normal and for Tris-washed chloroplasts. It is seen that in Tris-washed chloroplasts (Fig. 1B) the amplitude of the 334 nm absorption change is nearly the same as in normal chloroplasts (Fig. 1A), thus indicating that probably the primary electron acceptor X 320 of C_{II} is fully active also in Tris-washed chloroplasts. However, in Tris-washed chloroplasts the decay kinetics become faster (100—200 μs vs. 500 μs in normal chloroplasts). Beyond the fast decay also a slow phase ($\approx$ 20% of the total amplitude) is observed. Addition of System II electron donors (hydroquinone plus ascorbate) has no influence on the amplitude of the 334 nm absorption change (see Fig. 1D), but slightly increases its decay time ($\approx$ 300 μs). As in normal chloroplasts DCMU blocks nearly completely the absorption change at 334 nm also in Tris-washed chloroplasts (see Fig. 1C).

In order to prove that the absorption change at 334 nm in Tris-washed chloroplasts really reflects the reaction of X 320 some characteristic points of the difference spectrum have been measured. The results are given in Fig. 2. A comparison with the difference spectrum of van Gorkum [14] clearly shows that the observed absorption changes are caused by the redox reactions of X 320.

From these results it can be inferred that in Tris-washed chloroplasts there occurs either an internal charge recombination at the reaction centers themselves or a cyclic electron flow around System II including further internal electron carriers.

On the basis of kinetic measurements it should be possible to decide which mechanism is realized. If the observed X 320^- reoxidation would be caused mainly by an internal charge recombination of the type

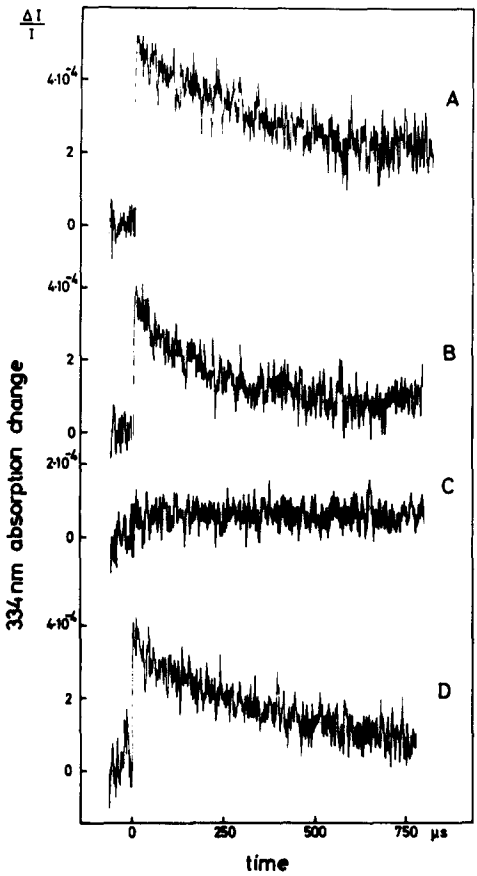

Fig. 1. Absorption changes at 334 nm as a function of time in normal (A) and in Tris-washed (B—D) chloroplasts of spinach. The reaction mixture contained: chloroplasts (10 μM chlorophyll), 250 μM $Na_3[Fe(CN)_6]$ as electron acceptor, 10 mM NaCl, 2 mM $MgCl_2$, 50 mM *N*-tris-(hydroxymethyl)methyl-glycine (Tricine)/NaOH, pH 7.2. Excitation with ultra short flashes, duration ≈ 500 ns, saturating intensity, time t_d between the flashes 160 ms. Optical pathlength 20 mm. Room temperature. (A) normal chloroplasts, (B) Tris-washed chloroplasts, (C) Tris-washed chloroplasts in presence of 2 μM DCMU, (D) Tris-washed chloroplasts in presence of 130 μM HQ + 1.3 mM ascorbate.

$C_{II}^- + \xrightarrow{k_{recomb.}} C_{II}$, then the dark recovery kinetics of X 320 and chlorophyll a_{II}, respectively, necessarily have to coincide. On the other hand, a kinetical coincidence should not arise for a cyclic electron flow including additional electron carriers beyond chlorophyll a_{II} and X 320.

The discovery of a 150—200 μs decay of chlorophyll a_{II}^+ in Tris-washed chloroplasts [8] favors the assumption of an internal charge recombination to be responsible for the X 320 recovery. However, recent findings [15] supporting evidence for the existence of a faster (35 μs) decay component of chlorophyll a_{II}^+ in Tris-washed chloroplasts which has no corresponding counterpart in the decay of $X\,320^-$ do not exclude the realization of a more complicated cyclic electron flow around System II. The experimental details together with a proposed reaction scheme will be discussed in a forthcoming paper (Gläser, Wolff and Renger, in preparation).

The present data are compatible with the suggestion of Rosenberg et al.

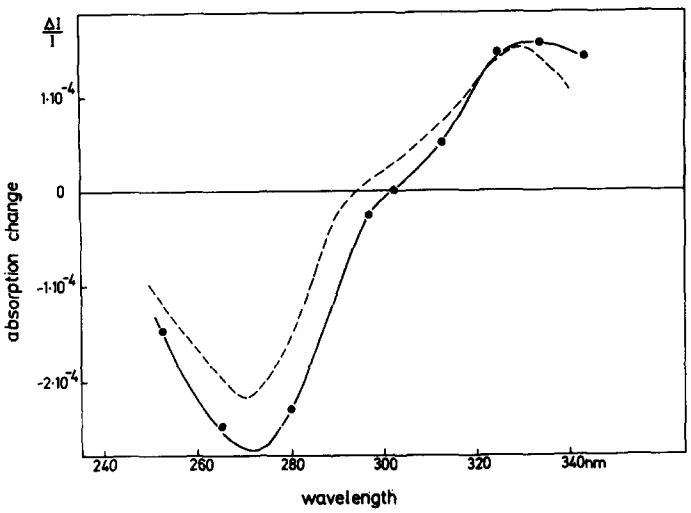

Fig. 2. Absorption changes with a decay kinetics of 100—200 μs as a function of wavelength in Tris-washed chloroplasts. Experimental conditions as in Fig. 1, except for chlorophyll concentration (100 μM) and optical pathlength (1 mm). Dotted line gives the difference spectrum of van Gorkum [14], normalized for comparison with the presented data at 325 nm.

[16] about the existence of a cyclic electron flow around System II in Tris-washed chloroplasts. The same conclusion has been drawn by Döring [8] on the basis of measurements of the 200 μs component of chlorophyll a_{II}. However, our results are not compatible with the proposed reaction schemes of refs. 8 and 16. It is clearly seen, that in contrast to the assumption in ref. 8 of a very fast recovery (< 20 μs) photoreduced X 320$^-$ is reoxidized really in 100—200 μs concomittantly with a slower recovery kinetics. This reoxidation is sensitive to DCMU. For the explanation of this effect it is indispensible to know the mechanism being responsible for the fast X 320 recovery. In the case of realization of internal charge recombination the effect of DCMU could be explained by the assumption that the positive charge(s) generated by the first turnover(s) is (are) not available for charge recombination, so that in the presence of DCMU the reaction centers of System II remain fixed in an inactive state.

If the observed fast X 320$^-$ reoxidation is caused mainly by a cyclic electron flow, several possibilities have to be considered. Though DCMU is known to interact also with the donor side of System II [17,18] this comparatively slow effect should not be responsible for blockage of a fast electron cycle in Tris-washed chloroplasts [16] for kinetic reasons. The inhibition by DCMU could be explained either by the assumption that the above-mentioned electron cycle also includes the secondary acceptor B [19] of System II or that DCMU blocks not only the electron efflux from X 320$^-$ into the plastoquinone pool, but in addition strongly decelerates any electron efflux from X 320$^-$ to the oxidizing side of System II. This second alternative would be in agreement with the slow ($\tau_{1/2} \approx 1$ s) electron cycle occurring in DCMU-inhibited chloroplasts [20,21]. Further experiments are required to clarify this question.

The existence of a fast dark recovery of System II makes C_{II} to be an

active photochemical trap even more effective for excitation energy consumpthan in normal chloroplasts because of its higher turnover rate. This explains the very low fluorescence yield in Tris-washed chloroplasts [3,4]. The fluorescence yield rises significantly in the presence of DCMU due to the inhibition of the rapid photochemical turnover rate. The high turnover rate also causes a rapid decay of the precursor state C_{II}^{-} of delayed luminescence [22]. Hence, one would anticipate a fast decay kinetics of delayed fluorescence in Tris-washed chloroplasts in the μs range. This effect remains to be investigated.

The fast photochemical turnover probably prevents a rapid light induced destruction of bulk pigments in Tris-washed chloroplasts by the very strong oxidizing equivalents of C_{II}. However, a complete protection seems not to be reached as is shown by the comparatively slow pigment bleaching in Tris-washed chloroplasts [4]. It would be interesting to speculate whether similar dissipative photochemical turnover routes at System II also exist in vivo in order to prevent rapid radiative pigment destruction in organisms with functional intact C_{II} but impaired water-splitting enzyme system Y (e.g. low fluorescence algal mutants [23]) or in greening organisms in which the reaction center C_{II} function is established before the development of full system Y activity [24].

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