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KINETICS OF REDUCTION OF THE PRIMARY DONOR OF PHOTOSYSTEM II

INFLUENCE OF pH IN VARIOUS PREPARATIONS

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Summary

The kinetics of reduction of P-680, oxidized by a flash, have been measured with chloroplasts treated with Tris, hydroxylamine or cholate, with PS-II particles from *Phormidium* (Tris-treated) and with subchloroplast particles prepared with digitonin or Triton. In all cases the electron transfer from D_1 to $P\text{-}680^+$ has similar rates, influenced similarly by pH and D_1 has a one-electron capacity.

The molecular species involved in photosynthetic oxygen evolution have not yet been identified. For this process the primary charge separation occurs in the reaction center of Photosystem II, where the primary donor P-680 is oxidized in a photochemical reaction. Oxidized P-680 is then re-reduced by a donor D_1, of unknown chemical nature, in a time of less than 1 μs [1,2]. The rate of electron donation from D_1 to $P\text{-}680^+$ is slower when oxygen evolution has been inhibited. The properties of D_1, in chloroplasts treated with Tris to inhibit oxygen evolution, have been compared with those of a species which gives the EPR Signal II_f [3,4]. This led to the proposal that the same species is

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Abbreviations: Mes, 2-(*N*-morpholino)ethanesulphonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

active in physiological conditions and after inhibition with Tris, albeit at a slower rate [5].

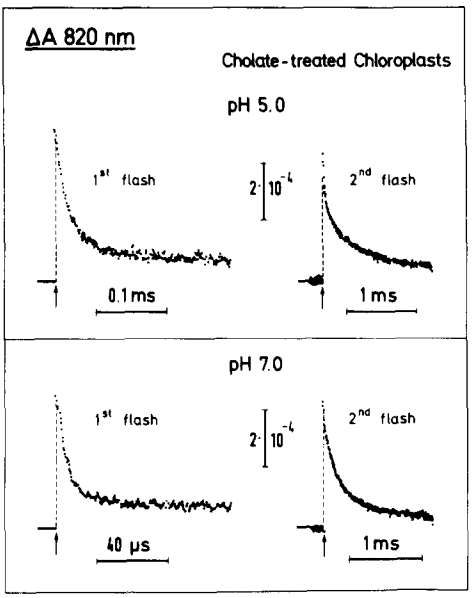
Recently, it has been shown that after Tris-inhibition the rate k_d of electron donation from D_1 to P-680$^+$ is strongly affected by pH, whereas the rate k_{br} of the back-reaction (electron return from the primary plastoquinone PQ_I^- to P-680$^+$) is not [6].

This report describes a similar effect of pH for the reaction k_d, with nearly the same rates, in spinach chloroplasts inhibited in oxygen evolution by high concentration of alkaline Tris or of hydroxylamine or cholate and in subchloroplast particles prepared with the detergents digitonin or Triton. Photosystem II particles from a blue-green alga displayed the same kinetic behaviour after treatment with Tris.

Kinetics of absorption changes at 820 nm due to the oxidation of P-680 by a ruby laser flash were measured as previously described [5,6] at 21°C. Before the laser flash, the material was either kept in darkness for 1 min (dark-adapted) or preilluminated with one xenon flash (50 ms before the laser) or by xenon flashes fired repetitively at 1 Hz. Spinach chloroplasts were prepared and treated according to published procedures, with Tris [7], with hydroxylamine [8,9] or with cholate [10]. Subchloroplast particles from spinach were prepared with digitonin (D-10 particles, Ref. 11), or with Triton X-100 (TSF-II particles, Ref. 12). Photosystem II particles were prepared from *Phormidium laminosum* [13]. For treatment with Tris, they were suspended in 0.8 M Tris (pH 8.0), incubated for 20 min in the light and pelleted by centrifugation (4 h at 200 000 × *g*). The pellets of chloroplasts or of particles were suspended in a small volume of water and kept on ice. Before each measurement an aliquot of the sample was added to a solution containing 10 mM of KCl, 2 mM of $MgCl_2$ and 50 mM of buffer succinate (pH 4.0 or 5.0), Mes (pH 6.0), Tricine (pH 7.0) or Tris (pH 8.0 or 9.0). Potassium ferricyanide (2 mM) was added to the cuvette for all experiments unless otherwise mentioned.

In all the preparations after dark-adaptation, the flash-induced absorption increase at 820 nm, which measures the oxidation of P-680, is followed by a rapid decay. The decay is multiphasic with a major fast phase ($t_{1/2}$ smaller than 60 μs), and slower phases. Examples of the decay are presented in Figs. 1 and 2. The $t_{1/2}$ of the fast phase varies with pH in all of the preparations (see Table I). The experimental uncertainty for each sample varies from about 1 μs at pH 8.0 to 5 μs at pH 4.0. Additional fluctuations result with the sample preparation, especially for the subchloroplast particles. Two different preparations of D-10 particles gave the results shown in Table I. The reason for these variations may arise from the presence of a phase with a $t_{1/2}$ of 20—30 μs, with a fluctuating magnitude, as was observed in Tris-treated chloroplasts [5]. Several TSF-II particle preparations presented an additional problem: a very fast phase ($t_{1/2}$ 2—3 μs) which was independent of pH was present. Other experiments indicate [14] that this rapid phase reflects the formation and decay of the triplet state of chlorophyll *a* molecules. These triplets were formed in a negligible amount in the particle preparation used for the results in Table I.

The total size of the flash-induced absorption change varied little with pH although it was often higher (by about 20%) at the lowest pH. If the extinc-



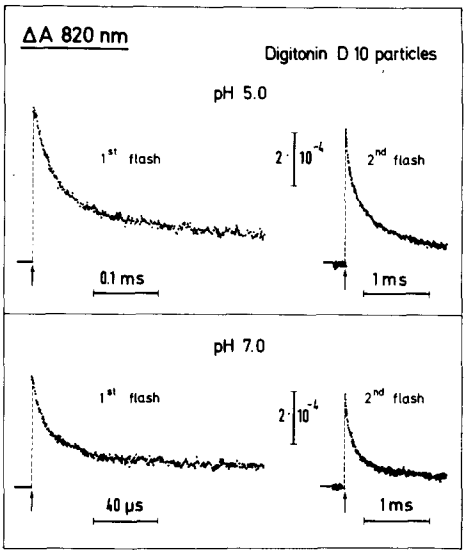


Fig. 1. Absorption change at 820 nm induced by a ruby laser flash in cholate-treated chloroplasts suspended in buffers at pH 5.0 and 7.0. The laser flash was preceded by a time of darkness (1st flash) or by a xenon flash (2nd flash). Chlorophyll concentration $3 \cdot 10^{-5}$ M. Average of two experiments.

Fig. 2. Same as Fig. 1, with D-10 subchloroplast particles. The chlorophyll concentration was $2.5 \cdot 10^{-5}$ M for the experiments at pH 5 and $2.0 \cdot 10^{-5}$ M for the experiments at pH 7.

tion coefficient of P-680$^+$ at 820 nm is assumed to be 7000 $M^{-1} \cdot cm^{-1}$, we determine one P-680 oxidized per 400—500 chlorophylls in treated chloroplasts, one per 300—400 in D-10 or TSF-II particles, one per 60 in Photosystem II particles from *Phormidium*.

Following preillumination of the spinach chloroplast preparations, the reduction of P-680$^+$ is slower (see Figs. 1 and 2), as found previously [5,6]. Nearly

TABLE I

HALFTIME OF THE FAST PHASE IN THE DECAY OF THE FLASH-INDUCED ABSORPTION INCREASE AT 820 nm, WITH DIFFERENT PREPARATIONS AT VARIOUS pH VALUES, AFTER DARK ADAPTATION

The data for Tris-treated chloroplasts are from Ref. 6. The two lines for D-10 particles correspond to two separate preparations. D-10 and TSF-II particles were prepared as described in the text; they were not treated with Tris. Each value is an average of 2—8 experiments.

Material	Halftime (μs)					
pH	4	5	6	7	8	9
Tris-treated chloroplasts	32	14	7.2	4.6	2.5	1.4
Cholate-treated chloroplasts	28	15	12	5.2	3.5	—
NH_2OH-treated chloroplasts	—	25	13	7.5	3.2	—
D-10 particles	40	20	11	7	3.0	—
	56	36	28	16	11	—
TSF-II particles	40	24	11	6	2.4	—
Tris-treated *Phormidium* particles	21	15	8.5	4.5	1.7	—

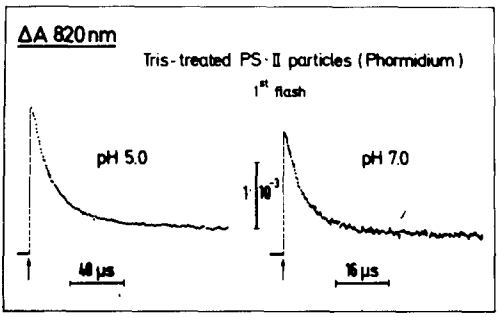


Fig. 3. Absorption change at 820 nm induced by a ruby laser flash in dark-adapted Tris-treated Photosystem-II particles from *Phormidium laminosum*, suspended in buffers at pH 5.0 or 7.0. Chlorophyll concentration $2.0 \cdot 10^{-5}$ M. Average of four experiments.

monophasic kinetics ($t_{1/2} \simeq 120$ µs) were obtained with cholate-, Tris- and hydroxylamine-treated chloroplasts at pH 6, 7 or 8, when the laser flash was preceded by one single xenon flash. With the other preparations and with cholate-treated samples at pH 4 or 5, the decay was multiphasic. However, the major phase was of intermediate $t_{1/2}$ (varying between 80 and 200 µs) and did not appear to vary significantly with either pH or with the material assayed. Photosystem II particles from *P. laminosum* constituted an exception since the $t_{1/2}$ of the slow phase was significantly larger: $\simeq$ 800 µs.

These results further confirm and extend the earlier finding [6] that the rate of electron transfer from the donor D_1 to P-680$^+$ is very sensitive to pH. The mechanism of this effect is not known; it may result from several states of protonation of D_1. The similarities of the effect of pH on this reaction and the similar $t_{1/2}$ support the hypothesis that the donation reaction to P-680$^+$ examined in all preparations is the same. This is especially noteworthy since chloroplasts treated with Tris, hydroxylamine or cholate have been often used in studies of electron transfer in Photosystem II. Their common properties, as to the donor side of Photosystem II, now permit helpful comparisons. For example, in these treated chloroplasts, P-680$^+$ is reduced slowly, in a back-reaction with PQ_1^-, after the second flash in the presence of ferricyanide. As proposed earlier for Tris-treated chloroplasts [5], this indicates that only one electron donor of high redox potential, with a one electron capacity, is associated with P-680 in chloroplasts treated with hydroxylamine or with cholate.

The rate of reduction of P-680$^+$ is also pH-dependent, with similar values, in subchloroplast particles prepared with digitonin or triton. This shows that the inferred donor D_1 is present and active in those particles. The pH dependence of the rate of electron donation to P-680$^+$ can be used as an analytical tool to differentiate the flash-induced formation of P-680$^+$ and of the triplet state of chlorophyll *a*, which often appears in subchloroplast particles. While the absorption properties of triplet chlorophyll *a* are similar to those of P-680$^+$, the lifetime of the triple is not influenced by the pH (data not shown). It is remarkable that the rates of transfer are similar and similarly influenced by pH, in the Photosystem II of higher plants and of blue-green algae. The electron transfer step from D_1 to P-680 thus appears to be highly stable with respect to the effect of chemicals and evolutionary drift.

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