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ABSORBANCE CHANGES DUE TO THE CHARGE-ACCUMULATING SPECIES IN SYSTEM 2 OF PHOTOSYNTHESIS

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SUMMARY

Absorbance changes are reported associated with Photosystem II and showing a periodicity of two and four as a function of flash number.

The absorbance changes showing a periodicity of two were found to occur in the presence of artificial electron donors as well and are presumably caused by the secondary electron acceptor R of Photosystem II. The absorbance difference spectra suggest that R is a plastoquinone molecule, which is reduced to its semiquinone anion after an uneven number of flashes. After an even number of flashes, the semiquinone is reoxidized. The absorbance changes showing a periodicity of four are tentatively ascribed to the charge accumulating donor complex of Photosystem II.

INTRODUCTION

There is now ample evidence that charge-accumulating mechanisms function both at the oxidizing and the reducing side of Photosystem II. According to the model of Kok et al. [1], four positive charges have to be stored at the donor side of Photosystem II before water oxidation takes places. In a series of saturating flashes, spaced at less than a few seconds, the successive charge-accumulation states (the so-called S-states) are obtained and are stable in the dark for at least several seconds [2]. Recent experiments [3, 4, 5] have shown that at the acceptor side a charge-accumulation occurs as well, related to the simultaneous transfer of two electrons to the plastoquinone pool. Using dark adapted chloroplasts as the starting material, it was shown that after an uneven number of flashes a secondary electron acceptor R was mainly in the state R^-, whereas after an even number of flashes R^- was reduced to R^{2-}, which was rapidly reoxidized to R by the plastoquinone pool. The redox state R^- decays with a half time of 3 min [4].

Therefore if one or both these charge-accumulating species have different extinction coefficients in their different redox states one would expect absorbance

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

changes which show a periodicity of four or two with flash number, and which are stable in the dark between the successive flashes in the series.

We now report absorbance changes which indeed show a periodicity with flash number and which may be due to both charge accumulating mechanisms.

MATERIALS AND METHODS

Absorbance changes were measured with a single beam spectrophotometer. A timer-controlled flow system provided a fresh sample, kept in the dark for 15–60 min, about 8 s before each measurement. The sample was illuminated by a series of red flashes, spaced at 290 ms, of 4 μs duration and of saturating intensity. Light saturation was checked by measuring the fluorescence increase caused by a flash in the presence of 50 μM DCMU and 1 mM hydroxylamine. Suitable filters protected the photomultiplier from the actinic flashes. This report concerns only those flash-induced absorbance changes, which persisted during the dark interval between successive flashes; rapid transients, (during the first 30 ms after a flash) are disregarded. Chloroplasts were prepared from freshly cut spinach leaves as described elsewhere [6] except that the buffer used contained sorbitol instead of sucrose.

RESULTS

Fig. 1A shows the amplitude of absorbance changes at 310 nm induced by a series of flashes. A damped oscillation with a periodicity of four with flash number occurred, apparently superimposed on a positive change occurring at all flashes. The

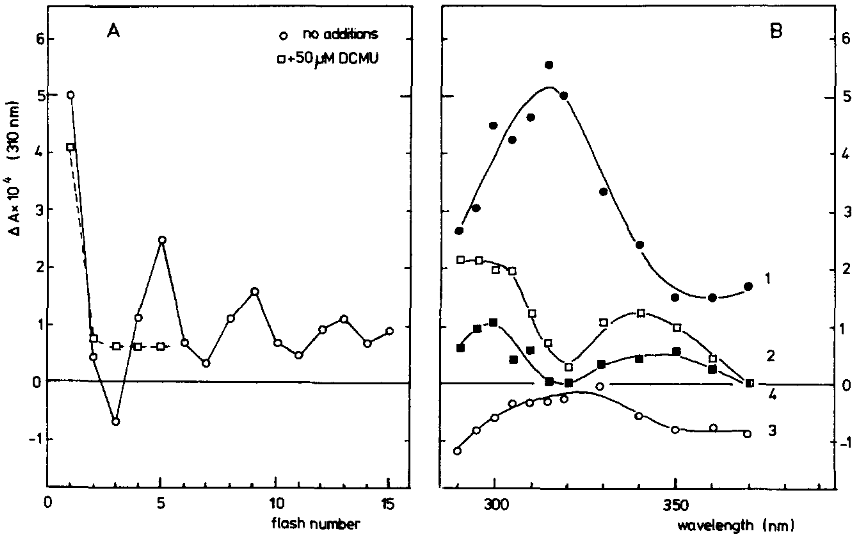

Fig. 1. (A) Absorbance changes at 310 nm induced by a series of short saturating flashes in the absence (circles) and presence (squares) of 50 μM DCMU. Optical pathlength 1.2 mm; chlorophyll concentration 150 μM. Average of 16 (circles) or 4 (squares) measurements. (B) Absorbance difference spectra caused by the first four flashes. Conditions as in Fig. 1A.

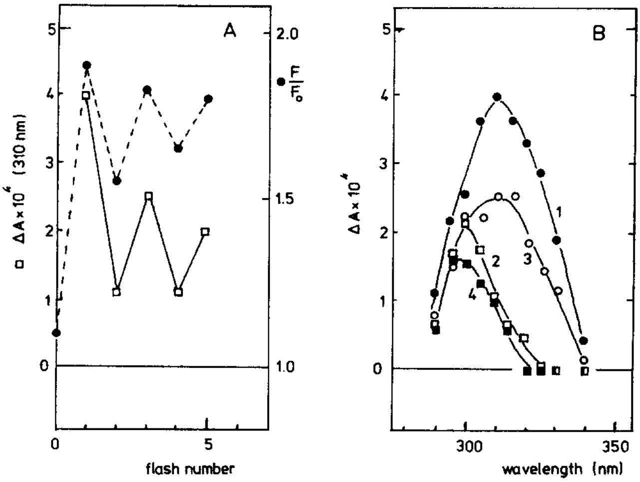

Fig. 2. (A) Absorbance changes at 310 nm induced by a series of short saturating flashes in the presence of 1 mM hydroxylamine (squares). Further conditions as in Fig. 1A. Circles: increase of fluorescence around 680 nm induced by the addition of 50 μM DCMU. (B) Absorbance difference spectra caused by the first four flashes in the presence of 1 mM hydroxylamine. Conditions as in Fig. 1A.

disappearance of the flash number dependence after the first flash in the presence of DCMU indicates that the oscillating absorbance changes are due to Photosystem II.

The difference spectra induced by the first four flashes are shown in Fig. 1B. The most marked feature in the spectra is an absorption band around 320 nm, which appears after an uneven number of flashes and disappears again on even flash numbers. This change with a periodicity of two with flash number seems to be superimposed on changes with a periodicity of four, which apparently have a rather flat difference spectrum in the wavelength range studied.

Redox reactions of the cytochromes did not contribute to the oscillating absorbance changes. In measurements with sufficient sensitivity to have detected the oxidation or reduction of one cytochrome per 4000 chlorophylls, no oscillating absorbance changes were observed in the green and blue parts of the spectrum.

At concentrations known to inhibit oxygen evolution the artificial system-2 electron donors hydroxylamine [7] and tetraphenylboron [8] were found to suppress completely the absorbance changes with a periodicity of four, but not those with two-fold periodicity (Fig. 2A). This supports the assignment of only the changes with a periodicity of four to charge accumulation on the oxidizing side of Photosystem II, while the two-fold periodicity is apparently not related to the S-states. More information will be needed to identify the component(s) which cause the absorbance induced by charge accumulation on the oxidizing side of Photosystem II and which show a periodicity of four upon flash number.

Fig. 2A also shows the amplitude of the fluorescence increase induced by addition of DCMU after a varying number of flashes under the same conditions. The oscillation of this amplitude with flash number reveals the same two-fold periodicity as observed by Velthuys and Amesz [4] in Tris-washed chloroplasts using a slightly different experimental procedure, which reflects charge accumulation on R, the secondary electron acceptor of system 2. It seems likely that the absorbance changes with two-fold periodicity are related to this charge accumulation.

The difference spectra induced by the first four flashes in the presence of hydroxylamine are shown in Fig. 2B. They appear to be composed of an increase around 310 nm occurring at each flash (in first approximation equal to the mean of the spectra induced by a pair of successive flashes) and the alternating appearance and disappearance of a band around 320 nm (in first approximation equal to half the difference of the spectra induced by a pair of successive flashes). The peak wavelength and the width of this latter band were within experimental error identical to that of anionic plastosemiquinone in methanol [9], suggesting that R^- may be identified as such. Since the fluorescence yield was low between flashes and independent of the flash number, Q^-, which has also been identified as a plastosemiquinone anion [10], contributed only to the transient absorbance changes which are not discussed in this report. The initial amplitude of the oscillation with period 2 could be accounted for by one plastosemiquinone per 960 chlorophyll molecules, assuming a differential extinction coefficient at 320 nm of 12 $mM^{-1} \cdot cm^{-1}$ [9], a flattening factor of 1.5 [11] and 10 % misses on each flash. This concentration is only 40 % of the estimated system 2 reaction center concentration, which could be explained by the presence of R^- in 30 % of the centers in dark adapted chloroplasts. Velthuys [12] also concluded from measurements of the dithionite induced fluorescence increase that an appreciable part of R is in the state R^- in dark-adapted Tris-washed chloroplasts.

Stiehl and Witt [13] using the repetitive flash technique observed absorbance changes around 320 nm which decayed with a half time of 0.6 ms. They attributed these absorbance change to the primary acceptor Q. Since Q has also been identified as a special plastoquinone molecule, which by the primary photoact is reduced to a semiquinone anion [10], the reoxidation of Q^- by R may not cause absorbance changes. The reoxidation of Q^- by R^-, however, might very well contribute to the absorbance changes with a half time of 0.6 ms, as reported by Stiehl and Witt [13].

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