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IDENTIFICATION OF THE REDUCED PRIMARY ELECTRON ACCEPTOR OF PHOTOSYSTEM II AS A BOUND SEMIQUINONE ANION

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SUMMARY

The complete absorption difference spectrum of the primary electron acceptor of Photosystem II has been measured at room temperature in subchloroplast fragments prepared with deoxycholate. The shape and amplitude of the spectrum indicate that the primary reaction involves the reduction of one bound plastoquinone molecule per reaction center to its semiquinone anion. In addition two small absorbance band shifts occur near 545 (C550) and 685 nm, which may be due to an influence of the semiquinone on the absorption spectrum of a reaction center pigment.

The photoreduction of the primary electron acceptor Q of Photosystem II is known to be accompanied by an increase of the chlorophyll fluorescence yield [1] and by a blue shift of a small absorption band at 546 nm, designated C550, (for a review, see ref. 2). We have recently [3] demonstrated the occurrence of both phenomena in subchloroplast fragments prepared with deoxycholate according to the method of Bril et al. [4]. Making use of both effects it was shown that in these particles Q is largely in its reduced state. After oxidation its photoreduction could be measured. Its reoxidation in the dark was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), except in the presence of 1,1'-diphenyl-2-picrylhydrazyl (DPPH). We now report absorbance changes which reflect these same properties and can be ascribed to one bound plastoquinone molecule per reaction center, which is photoreduced to the plastosemiquinone anion.

The particles were obtained as the $10\,000 \times g$ pellet after deoxycholate treatment of spinach chloroplasts ("DOC 2 particles") and measurements were carried out at room temperature as described previously [3], unless otherwise indicated. The signal to noise ratio was enhanced with a signal averager.

Fig. 1 shows the kinetics of absorbance changes correlated with C550: after addition of ferricyanide the light-induced changes can be measured (A). DCMU, added after ferricyanide, does not inhibit the light-induced change but only its dark decay. Complete irreversibility is obtained if the electron donor tetraphenylboran is

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

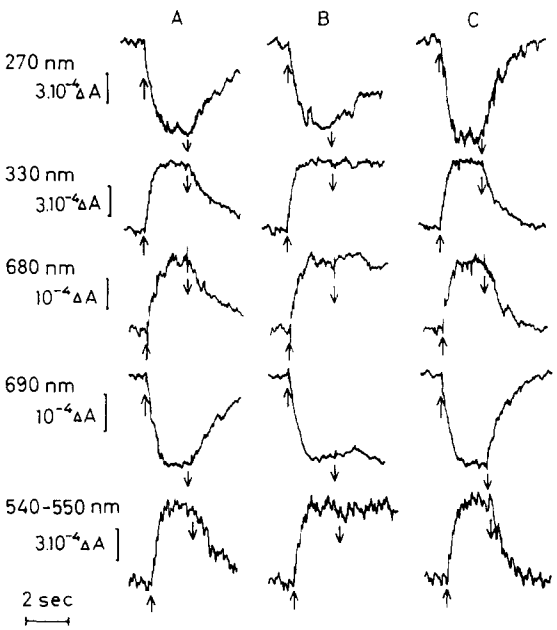

Fig. 1. Kinetics of absorbance changes in the presence of (A) 50 μM ferricyanide, (B) 50 μM ferricyanide, 5 μM DCMU and 10 μM tetraphenylboran (omitted at 270 nm because it induces additional absorbance changes) and (C) 5 μM DCMU and 5 μM DPPH. Each curve is the average of ten measurements with a chlorophyll concentration of 150 μg/ml in a 1 mm cuvette. P700 changes were prevented by far red background light ($\lambda > 710$ nm). The bottom graphs are single measurements of the 540 minus 550 nm difference in a 1 cm cuvette with a chlorophyll concentration of 75 μg/ml and the same concentrations of additions. Upward and downward arrows mark the onset and offset of actinic light.

added as well to prevent back reaction (B). After addition of DPPH the change is rapidly reversible even in the presence of DCMU (C). The complete light minus dark difference spectrum was measured from 250 to 720 nm and the significant parts are shown in Fig. 2. The ultraviolet part is similar to the plastosemiquinone anion minus plastoquinone difference spectrum measured by Bensasson and Land [5] (dashed line), but appears to be shifted by about 15 nm to longer wavelengths. Such a shift was found for the difference spectrum of the reduction of ubiquinone to the ubisemiquinone anion in bacterial reaction centers as well [6] and is probably due to the tight binding of the quinone in the reaction center. The extinction coefficients in Fig. 2 were calculated assuming a concentration of one molecule per reaction center and appear to agree well with the data of Bensasson and Land. The concentration of reaction centers was 1 : 300 chlorophyll molecules, determined on the basis of both C550 and P680 measurements [3]. Absorbance changes near 320 nm have previously been attributed to the primary electron acceptor of photosystem II and tentatively been ascribed to plastosemiquinone formation [7, 8]. The difference between the spectrum measured by Stiehl and Witt and our measurements may be due to a contribution of the secondary acceptor, which does not occur in our measurements.

In addition to the absorbance changes caused by plastosemiquinone formation

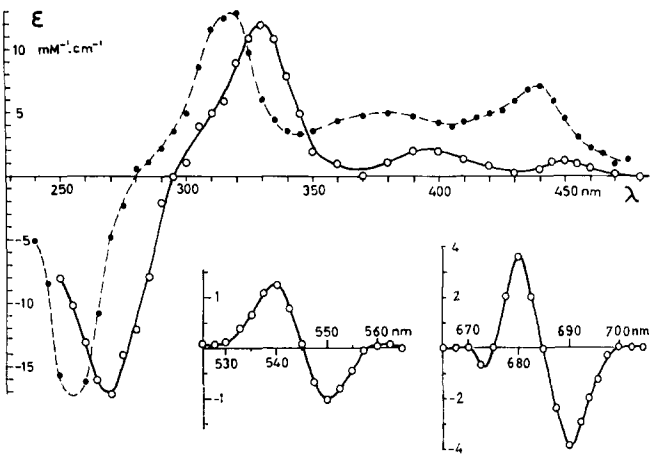

Fig. 2. Light minus dark difference spectrum of absorbance changes as shown in Fig. 1. Each point is the average of five measurements. P700 changes were avoided by keeping the dark time between measurements short. 5 μM DPPH was added, except for the 470 to 600 nm region where significant changes due to the reoxidation of reduced DPPH occurred. In this case 100 μM ferricyanide and 20 μM tetraphenylboran were added, using a chlorophyll concentration of 360 μM. Otherwise 250 μM chlorophyll was used. The optical path length was 1.1 mm and the half band width was 5 nm in the ultraviolet and 2 nm in the visible region. Extinction coefficients were calculated on the assumption of a one to one ratio of the quinone to C550 and to P680 (ref. 3). The dashed line is the plastosemiquinone anion minus plastoquinone difference spectrum from ref. 5.

itself there are other small changes in the green and red regions of the spectrum: the band shift due to C550 and a band shift centered at 685 nm. By analogy to similar changes in bacterial reaction centers these may be attributed to band shifts of reaction center pigments [9], which may be caused by an electrostatic influence of the semiquinone. Both changes could be due to a bound or aggregated form of pheophytin *a*. Like C550, the changes in the red region have been observed at low temperature as well and have been shown to reflect the reduction of the primary electron acceptor [10] and Visser, J. W. M., Amesz, J. and Rijgersberg, C. P., in preparation.

The absorbance changes reported here were not accompanied by significant electron spin resonance changes in the $g = 2$ region. This presumably is due to the special binding of the semiquinone in the reaction center.

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