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CORRELATION OF THE LIGHT-INDUCED CHANGE OF ABSORBANCE WITH ESR SIGNAL OF PHOTOSYSTEM II IN PRESENCE OF SILICOMOLYBDATE

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SUMMARY: The light-induced dark-reversible ESR signal in chloroplast fragments enriched in photosystem II and free from P700 contamination has been observed in the presence of silicomolybdate as an electron acceptor operating directly on the photosystem II primary acceptor. The signal at g = 2.0025 and with line-width ΔHpp = 9G rises and decays in close correlation with the photobleaching band centered at 680 nm and the minor peak at 435 nm.

It is widely accepted that the primary photochemical event in photosynthesis is the electron transfer from the reaction center chlorophyll to the primary acceptor. This charge separation is manifested in photosystem I (PS I) as the appearance of the light-induced ESR signal of the oxidised chlorophyll a radical cation. This is observable over a very large range of temperatures and other experimental conditions and correlated with photobleaching at 703 nm. In photosystem II, however, the situation is still not unambiguous /1/. Döring et al /2,3,/ observed spectral changes centered at 680 nm attributable to the reaction center chlorophyll a of PS II. Haveman and Mathis /4/ used the appearance of the absorbance band at 825 nm to observe P680 oxidation. Change in optical absorbance and ESR signals have been observed

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in deoxycholate treated preparations without detergent at low pH values /5,6/ and also at cryogenic temperatures /7,8/. Observations on P680 oxidation by means of ESR spectroscopy are mainly restricted by the short life-time of the oxidised P680. This is the result of several reactions leading to $P680^+$ reduction. Recently we have attempted to overcome this restriction by using silicomolybdate (SiMo) /9/ as an electron acceptor operating directly on the primary acceptor of PS II /10,11,12/. Results are now presented on the correlation of this light-induced dark-reversible ESR signal with the optical absorbance changes near 680 and 435 nm. These confirm our interpretation that the SiMo-dependent signal is associated with the radical cation of chlorophyll a in photosystem II.

MATERIALS AND METHODS

Chloroplast fragments of PS II were isolated from the leaves of 14 day old Vicia faba plants by a method of Anderson and Boardman /13/ as modified by Shutilova et al /14/. The preparation was about 40 fold purified on optical basis from P700 contamination as compared to chloroplasts. Its properties are described in detail elsewhere /14,15/. The ESR spectra have been recorded using Varian E4 equipment. Measurements of absorbance changes induced by continous illumination (650-1100 nm, up to 10 W m^{-2}) were made with a single beam difference spectrophotometer provided with a mechanical modulator for separation of the actinic and measuring light beams /16/. The samples were placed in a flat cell of 2 mm path-length. With such samples the instrument could be adjusted so as to avoid possible artifacts. The delayed light emission as well as fluorescence excited by a measuring beam ($\leq$ 0,05 W m^{-2}, band-width ~6 nm) were eliminated by ensuring a good spacing between the cell and the input of the phototube and by limiting the supply voltage to the phototube by 400-500 V.

RESULTS AND DISCUSSION

Fig.1 shows the ESR signal ($g \sim 2.0025$; ΔH_{pp} = 9.0G) in a suspension of PS II fragments in the presence of 10^{-3}M SiMo ($Na_4SiMo_{12}O_{40}$). The kinetics of its appearance and dark decay

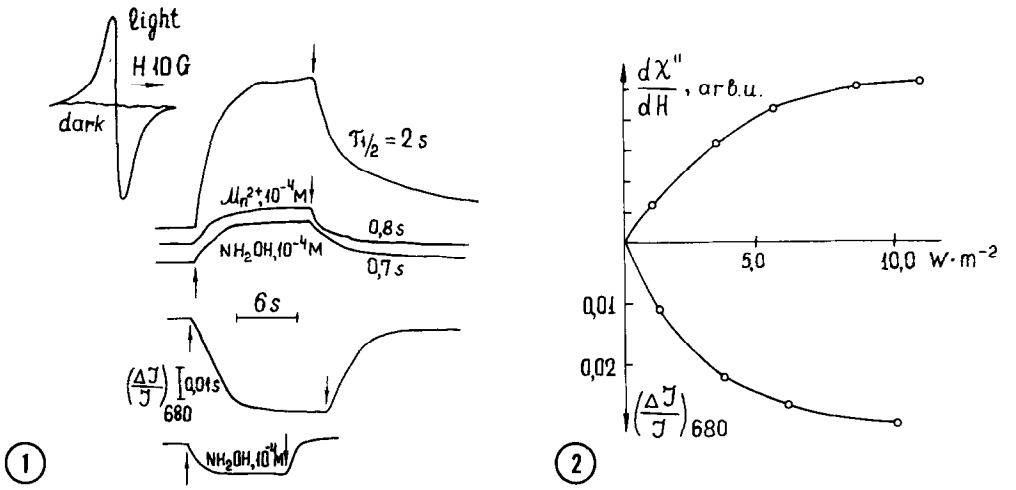

Fig.1. ESR signal in PS II fragments and the kinetics of its rise under saturating light (12W m^{-2}) and the subsequent dark decay (0,5 mg/ml chlorophyll, 10^{-3}M SiMo). The magnetic field corresponds to the low-field peak of the first derivative signal. The lower curves are the recordings of photobleaching at 680 nm under similar conditions except that the chlorophyll concentration has been adjusted to 0.25 mg/ml. All recordings at 0°.

Fig.2. The light saturation curves of the ESR signal and of the photobleaching at 680 nm. The same conditions as in Fig.1.

at near 0° is also indicated. The dark decay is biphasic with first-order rate constants k_1=0,05 s^{-1} and k_2=0,33 s^{-1}. At 0° the signal is readily saturated by light (Fig.2). It is also observable at room temperature but the light saturation is difficult to obtain.

Hydroxylamine, an electron donor of PS II, reduces the ESR signal and increases the rate of its dark decay as is shown in Fig.1. Some typical recordings of light-induced changes at 680 nm under saturating light are also shown in this figure. Fig.3 shows the spectrum of photobleaching with a major band centered at 680 nm and a minor peak at 435 nm obtained from such kinetic recordings. This spectrum agrees with the spectral data of Döring et al /2,3/ obtained from pulse kinetical measurments. Fi-

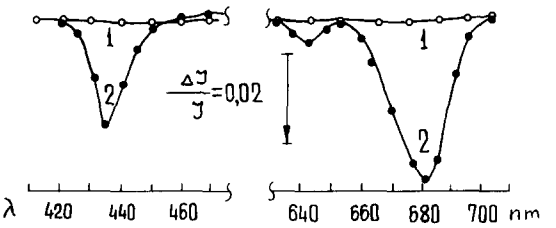

Fig.3. The spectrum of photobleaching. Only the dark-reversible part of the absorbance change in response to the first illumination has been measured. 1 - control without electron acceptor, 2 - in presence of SiMo. Other conditions the same as in Fig.1.

nally, Fig.2 (lower curve) shows the absorbance change at 680 nm as a function of light intensity similar to that of the ESR signal.

A comparison of all these observations clearly indicates the close correlation between the optical absorbance change and the ESR signal. The steady state photooxidation of chlorophyll a in photosystem II has been provided in these experiments by the use of silicomolybdate as an electron acceptor interacting directly with the primary acceptor of photosystem II /10-12/; thus the limiting step of the primary acceptor oxidation by the plastoquinone pool is avoided. As a result the oxidation effectively competes with the reverse reaction of charge separation. Under this condition together with the partial inhibition of the electron transfer on the oxidizing side of PS II in our preparations (not competent in O_2 evolution) there seems to be steady state oxidation of chlorophyll a in PS under moderate light in the physiological range of temperature and pH-values. No other electron acceptors, such as ferricyanide, dichlorphenolindophenol and methylviologen were effective in this respect. The molar extinction of the SiMo-dependent light-induced center has been evaluated by comparing the ESR signal with the absorbance changes as

described in detail elsewhere /17/ and has been found to be $E_{680} \simeq 0{,}66\ 10^5\ M^{-1}cm^{-1}$. This is very close to the value reported earlier for P700 in photosystem I particles /18/.

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