FURTHER EVIDENCE OF X-320 AS A PRIMARY ACCEPTOR OF PHOTOSYSTEM II IN PHOTOSYNTHESIS

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Properties of an unknown primary electron acceptor Q of light reaction II of photosynthesis in green plants has been studied by measurement of fluorescence changes by a number of authors [1–4]. Q is a one-electron acceptor which is different from a pool of secondary acceptors A. The oxidation Q^- by A can be blocked by the action of DCMU** [1]. This has recently been verified [5]. The reduction of Q in the presence of DCMU still occurs but the reoxidation islargely delayed. Fluorescence changes at liquid nitrogen temperatures indicate that reduction of Q takes place [3,6].

A light-induced difference spectrum in the ultraviolet has been discovered with maxima at 320 and 400 nm [7,8]. The substance responsible for these changes is termed X-320. The formation of X-320 has been interpreted as the reduction of a primary acceptor by chlorophyll-a_{II} [9]. X-320 is reoxidized by a pool of secondary acceptors identified with the pool of plastoquinone (PQ) [7,8]. The formation of X-320 takes place < 30 μsec. The reoxidation time of X-320 is 0.6 msec and is the same as the electron transfer time from water to plastoquinone measured by the O_2 yield in double flashes [10]. It is also identical with the reoxidation time of Q [11].

Two new experiments support that the oxidised form of X-320 acts as a primary acceptor of photosystem II.

Chloroplasts were prepared from market spinach

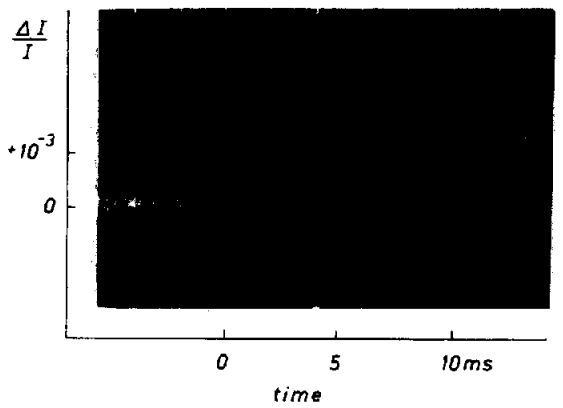

Fig. 1. Absorption changes of X-320 at $T = 140°K$, $\lambda = 334$ nm. Spinach chloroplasts suspended in sucrose syrup. Chlorophyll content 180 μg/ml, electron acceptor ferricyanide 2×10^{-4} M, sucrose 2.3 M, optical path length 0.12 cm, excitation: duration 2×10^{-5} sec, measuring light: grating monochromator, half-width 2 nm; intensity of measuring beam 10 μW/cm^2; electrical band width 30–500 Hz.

by the method of Winget et al. [18]. The sample consisted of chloroplasts, chlorophyll content 180 μg/ml; 2×10^{-4} M ferricyanide; sucrose approx 2.3 M in 0.05 M tricine–buffer, pH 7.2. This is frozen in the dark for low temperature measurements. The high sucrose concentration serves for full transparency at low temperatures, while at high temperatures it does not affect amplitude and decay kinetics of X-320 appreciably.

A flash-photolytic apparatus has been used for measurement of absorption changes equipped with compensation amplifier for detection of irreversible absorption changes at low temperature [19]. The measuring beam is switched on about 0.2 sec before a

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

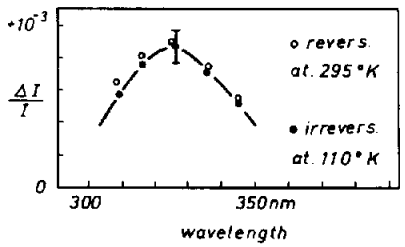

Fig. 2. Spectrum of irreversible absorption changes at T = 110°K (•-•-•). Spectrum of reversible absorption changes of X-320 at 295°K according to [7] computed on the basis of the chlorophyll concentrations used here (o-o-o).

flash is fired. The intensity of the measuring beam was up to 10 μW/cm^2, the intensity of the exciting flash was just saturating photosynthesis.

Fig. 1 shows the flash induced absorption change of X-320 at λ= 334 nm and T= 140°K. The amplitude corresponds to the amplitude at room temperature. The change at 140°K is completely irreversible (therefore pre-illumination by the measuring beam must be kept small). The irreversible changes at T= 110°K as function of wavelength is shown in fig. 2. For comparison also the spectrum of X-320 at 295°K is drawn (calculated from [7] for the higher concentrations used here). The agreement between both spectra is good. (It should be mentioned, that at wavelengths different from 335 nm some unidentified reversible changes are superimposed on the irreversible ones at 110°K).

Fig. 3 shows the action of DCMU on X-320 at room temperature. If DCMU is added to the chloroplasts the first flash produces X-320 in full (top of fig. 3). If, however, the pre-illumination period of the measuring beam (intensity 10 μW/cm^2) is about 20 sec or if a second flash is fired only after about 1 sec, the amplitude of the absorption change is zero (bottom of fig. 3). The same signal as in the first flash can be caused by a second flash, if a dark time of about 30 sec separates the flashes (during the dark time the measuring beam is switched off), indicating that DCMU does not inhibit the production of X-320 but delays the oxidation time strongly.

Both results show agreement of properties of X-320 with those of Q^- so far known from fluorescence measurements [1,3,5,6,]. This supports the interpretation that X-320 is a primary acceptor of photosystem

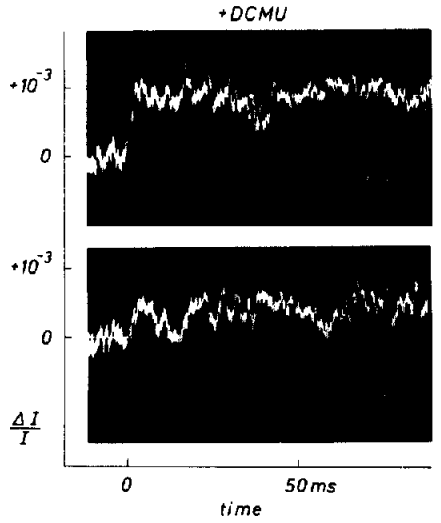

Fig. 3. Absorption changes at λ = 334 nm with addition of DCMU (T = 295°K). Top: Flash fired 0.2 sec after switching on the measuring beam; sample kept in darkness before. Bottom: Second flash fired after some 20 sec-illumination by the measuring beam (equal to a second flash fired after 1 sec). Concentration of DCMU: 2 × 10^{-6} M.

II. The DCMU experiment is consistent with the following model: DCMU blocks electron flow between X-320 and PQ. Q can be reduced by a flash and is reoxidized slowly probably by an oxidized product of the water splitting side of photosystem II. Such a model has been derived before from the behaviour of flash-induced H_2O oxidation in the presence of DCMU (A. Bennaun, 1971, Nature New Biol. 235, 5). On the other hand the site of DCMU action has been shown to be also at chlorophyll-a_{II} [20]. Therefore one has to assume two different sites of DCMU action.

A compound C550 [6,12-14,17] has been propozed as a primary acceptor of Chl-a_{II} [6,12]. At low temperature irrevesible absorption changes have been observed in the wavelength region around 558 nm, but at room temperature no flash-induced absorption changes are observed with kinetics which are expected for the primary acceptor (fast rise < 30 μsec, decay 0.6 msec) X-320 is therefore not identical with C550.

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