LIGHT-INDUCED ABSORPTION CHANGES IN THE NEAR ULTRAVIOLET OF THE PRIMARY ELECTRON ACCEPTOR OF PHOTOSYSTEM II AT LIQUID NITROGEN TEMPERATURE

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1. Introduction

Absorption changes near 320 nm in chloroplasts were first observed by Stiehl and Witt [1,2] and interpreted as to be due to an acceptor of Photosystem II. Much evidence has been presented that these absorption changes are due to the primary electron acceptor of Photosystem II, which is responsible for the quenching of chlorophyll fluorescence in its oxidized form [3] and identified in its reduced form as a plastosemiquinone anion radical [4–6]. Absorption changes near 550 nm due to the so-called 'C-550' were attributed to the primary acceptor [7,8] as well as the changes observed in the red region [5,9,10]. Efforts were made to show the correlation between the changes near 320 nm and 550 nm. Van Gorkum [5] saw similarity in the kinetics at the different wavelengths in desoxycholate-treated chloroplast fragments at room temperature and proposed that the absorption changes in the green and red regions were due to an electrostatic influence of the semiquinone on (a) neighbouring pigment molecule(s), possibly pheophytin a. Amesz et al. [6] working with chloroplasts at –40°C, observed similarity in kinetics of the absorption changes at 320 nm, of C-550 and of the Photosystem II component of the 515 nm absorption change. We report here on absorption changes at liquid nitrogen temperature induced by a laser flash or by continuous light. We find that under these conditions the absorption changes around 320 nm behave in the same way as C-550.

2. Materials and methods

Spinach chloroplasts were prepared in 20 mM Tricine buffer, pH 8.0, 10 mM NaCl and 0.4 M sucrose. They were stocked in liquid nitrogen in a mixture of buffer and glycerol (40–60 v/v). For the experiments the chloroplasts were further diluted in the glycerol mixture. Measurements of flash-induced absorption changes at –170°C were done as described in ref. 11, except for the following details. The photomultiplier (EMI 9656 QR) was protected by a Schott UG 11 filter and by two cuvettes containing respectively a solution of cryptocyanine in methanol and $CuSO_4$ in water. Before falling on the sample cuvette the measuring beam passed a monochromator (HUET M25, France) and a Schott UG 11–1 mm filter. In order to avoid actinic effects it was allowed to fall on the sample only for 0.25 sec during each experiment, using a photographic shutter. The laser flash passed a MTO 694-nm narrow band interference filter. Absorption changes induced by continuous light were measured as described in ref. 12.

3. Results and discussion

The laser flash-induced absorption change at 320 nm is shown in fig.1. A large part of the change appears to be reversible with a halftime of approx. 3.0 msec; about 25% is irreversible after the first flash. Within a series of successive flashes on the

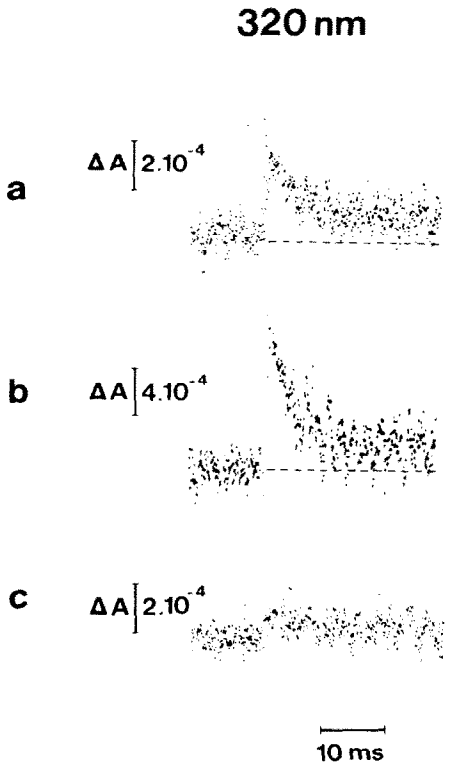

Fig.1. Laser flash-induced absorption changes in a chloroplasts suspension, at 320 nm (half-band width 12 nm), at −170°C. Optical path of the cuvette : 1 mm. (a) Changes following the first flash; (b) sum of the changes induced by the first four flashes; (c) changes induced by one flash after a saturating preillumination at −170°C. Concentration of chlorophyll : 0.35 mg·ml^{-1}. Average of 10 experiments.

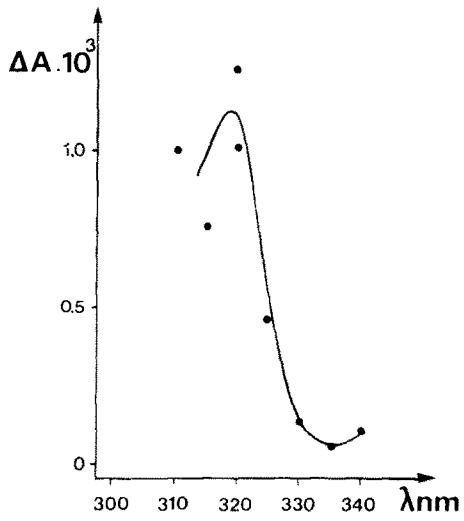

Fig.2. Spectrum of the changes in absorption (ΔA_{max}) in the near ultraviolet. The data are taken fron the sum of the first four flashes (half-band width 3 nm). Average of 5 experiments. The absorption change at 320 nm corresponds to an extinction coefficient of approx. 13 mM^{-1} cm^{-1} (the estimation is made assuming one molecule per cytochrome b559 oxidized by continuous light, at −170°C, and an extinction coefficient of 20 000 at 556 nm for reduced cytochrome b559). Concentration of chlorophyll : 0.35 mg·ml^{-1}

same sample there is a progressive decrease of both the reversible and the irreversible parts of the absorption change. The total absorption change due to the first four flashes is shown in fig.1. After a saturating preillumination at low temperature a flash induces only a small absorption change. This already suggests that the absorption change is mainly due to Photosystem II since Photosystem I absorption changes are reversible for about 40%. A more precise evidence for the attribution to Photosystem II is that in the presence of 10 μM DCMU plus 100 μM hydroxylamine, after a preillumination just prior to cooling no detectable signal was observed (not shown). This property has been found previously for C-550 [11,13]. The difference spectrum of the absorption change is shown in fig.2. Because of an important absorption by the filters, it was not possible to perform any measurement at wavelengths shorter than 310 nm. The difference spectrum of fig.2 fits fairly well with the one obtained in vitro for plasto-semiquinone anion minus plastoquinone [4] and the one obtained in vivo at −40°C by Amesz et al. [6].

On the same batch of chloroplasts we measured the flash-induced absorption changes at 820 nm (fig.3). The rapidly ($t_{1/2}$ = 3.0 msec) decaying phase of the absorption change has been attributed to Chl^{+}_{II}, the oxidized form of the primary donor chlorophyll in Photosystem II [11] (see also ref. 14). It must be pointed out that the irreversible part of the absorption change in fig.3 is not comparable with the irreversible part of the change at 320 nm, as it is unaffected by the treatment with DCMU plus hydroxylamine and preillumination. It has been

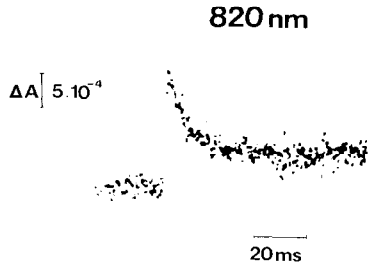

Fig.3 Flash-induced absorption changes at 820 nm. Average of the five first flashes at −170° C (half-band width 3nm). Optical path of the sample cuvette : 1.4 mm. Concentration of chlorophyll : 1mg·ml^{-1} (from the same batch as for figs. 1 and 2). Average of 5 experiments.

attributed to the oxidized form of P 700 [11]. The reversible parts in the absorption changes at 320 nm (fig.1) and 820 (fig.3) can be interpreted in terms of the back-reaction model [15,16] as previously done for C-550 and Chl_{II}^{+} (11,13). In fig.4, the absorption changes at 540 and 320 nm are shown upon continuous illumination. Also under these light-limiting conditions where dark reactions might interfere, the kinetics of the changes at both wavelenghts are the same, an observation which further confirms the above reported ones.

These results support the conclusion that absorption changes near 320 nm as well as those due to C-550 are both closely related to the reduction of

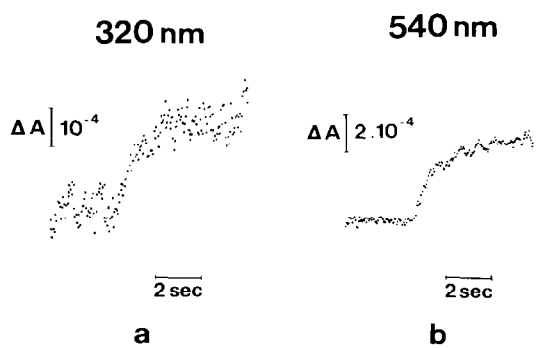

Fig.4. Absorption changes induced by continuous light, under light-limiting conditions, at 320 nm and 540 nm, at −170° C. (a) change at 320 nm. Half-band width 12 nm. Average of 10 experiments. (b) change at 540 nm. Half-band width 1 nm. Average of 5 experiments. Optical path of the sample cuvette : 1 mm. Concentration of chlorophyll : 0.25 mg·ml^{-1}.

the primary acceptor of Photosystem II. A unique property of Photosystem II at low temperature is that the primary photochemical reaction is totally effected by one short saturating flash, but is followed by an important back-reaction in about 3 msec. In a previous article [17], it has been reported that the reduction of plastoquinone at low temperature was irreversible after a short flash; the author mentioned some reversible absorption changes in the near ultraviolet but apparently overlooked their importancy in the interpretation. Our present results give further support to the assignement of plastoquinone as primary acceptor in Photosystem II and allow a coherent description of the behaviour of this Photosystem under flash excitation at low temperature.

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