PHOTOREDUCTION OF PLASTOQUINONE TO PLASTOSEMIQUINONE IN SPINACH CHLOROPLASTS AT −40°C

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1. Introduction

During the last years, light-induced absorbance changes related to the primary photoreaction of system 2 of photosynthesis have been studied in several laboratories. Absorbance changes near 545 nm (attributed to a compound named C-550 [1]), as well as in the ultraviolet region [2–4] have been attributed to the primary electron acceptor. Recent experiments of Van Gorkom [5] with spinach chloroplasts treated with the detergent deoxycholate indicate that the primary photoreaction involves the reduction of plastoquinone to its semiquinone anion, causing absorbance changes in the region 250–400 nm, and band shifts of a pigment or pigments absorbing near 545 nm (C-550) and 685 nm (see also ref. [6]).

Experiments with chloroplasts in the temperature region −40 to −90°C showed a dependence of the photochemical reaction on the number of positive charges in the pathway to water (the so-called S-state [7]) produced by preillumination with short, saturating flashes before cooling. This was reflected, amongst other things, in the rate of increase of chlorophyll *a* fluorescence, and in the extent of the absorbance changes of C-550 upon illumination [8–12]. At present, these phenomena are only poorly understood. In this paper we shall report some experiments on absorbance changes in the ultraviolet region at −40°C. The results support the hypothesis that the primary acceptor of photosystem 2 is plastoquinone, and show that its reduction, in agreement with the data on C-550, is dependent on the S-state of the reaction center.

2. Materials and methods

Chloroplasts, prepared as described in ref. [1], were suspended in a buffer of pH 7.8, containing 0.05 M *N*-tris(hydroxymethyl)methylglycine (Tricine), 0.01 M KC1, 0.002 M $MgC1_2$ and 0.4 M sucrose.

Measurements of absorbance changes were carried out on a similar splitbeam spectrophotometer as described by Amesz [13]. Dependent upon the wavelength of measurement an incandescent or a hydrogen lamp was used as light source. Suitable glass or liquid filters protected the photomultiplier from the actinic illumination. A second photomultiplier was used to monitor simultaneously the chlorophyll fluorescence, excited by the actinic light. Saturating flashes (8 μsec) were obtained from a xenon flash tube. The filter combinations used to filter the actinic light, the fluorescence and the flash light were the same as described in ref. [11]. Just before each measurement glycol was added to the dark-adapted chloroplast suspension to a final concentration of 53% v/v. The suspension was transferred to a thermostated measuring cuvette, optical path length 0.14 cm, equipped with quartz windows and cooled as described in ref. [14]. Preilluminating flashes were given at 0°C at 1 sec intervals. A temperature of −40°C was reached within 2 min after the flashes.

Chlorophyll concentrations were determined as described by Whatley and Arnon [15].

3. Results and discussion

Fig. 1 shows the kinetics of the light-induced absorbance changes at 320 nm at −40°C with different

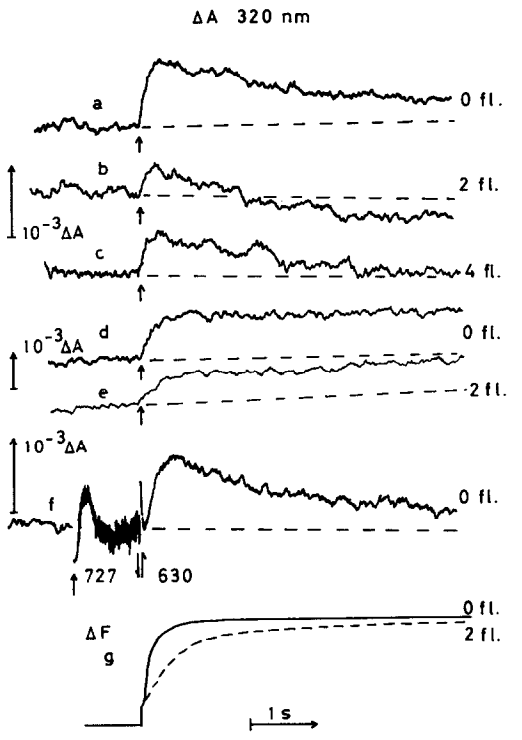

Fig. 1. Tracings a–f: absorbance changes at −40°C at 320 nm. Upward and downward pointing arrows mark the illumination periods. The intensities of actinic light were 13 nEinstein $cm^{-2}s^{-1}$ at 727 nm (sufficient to oxidize most of P700) and 3 nEinstein $cm^{-2}s^{-1}$ at 630 nm. The transients and noise during the 727 nm illumination are due to imperfect shielding of the photomultiplier from the actinic light. Tracings d and e: 2.5 mM ferricyanide was added; note the different sensitivity for these tracings. Chlorophyll concentration was 0.25 mM. Tracing g: Fluorescence of chlorophyll *a;* solid and broken line: conditions as for tracings a and b, respectively.

numbers of preilluminating flashes. The tracings show two phases, a fast increase in absorbance followed by a slow decrease. The rapid phase showed a 4-fold periodicity, with a minimum after two and maxima after four flashes and without preillumination (similarly as observed earlier for the rate of fluorescence increase and for absorbance changes at 518 nm and of C-550 [8,11]). Both the fast and the slow absorbance changes showed first order exponential kinetics. In the presence of ferricyanide the slow phase was absent and the maximum extent of the absorbance change was larger. This difference in magnitude might be accounted for by the absence of the slow phase. Tracing f of fig. 1 shows that the kinetics were not affected by a preceding illumination with far-red light, indicating that the absorbance changes are due to photosystem 2. This was confirmed in an experiment in which the chloroplasts had been preilluminated with 12 flashes in the presence of 10 mM hydroxylamine and 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in order to destroy photosystem 2 activity [16,17]. In this case no absorbance changes could be seen at 320 nm.

Comparison with the fluorescence kinetics (traces g and h) shows that for dark-adapted chloroplasts the fast phase and the fluorescence increase had nearly identical kinetics. After two flashes preillumination the rise time of the rapid absorbance increase seemed to be somewhat longer (visible when ferricyanide was added), whereas that of the fluorescence was much more increased and different from that of either absorbance change.

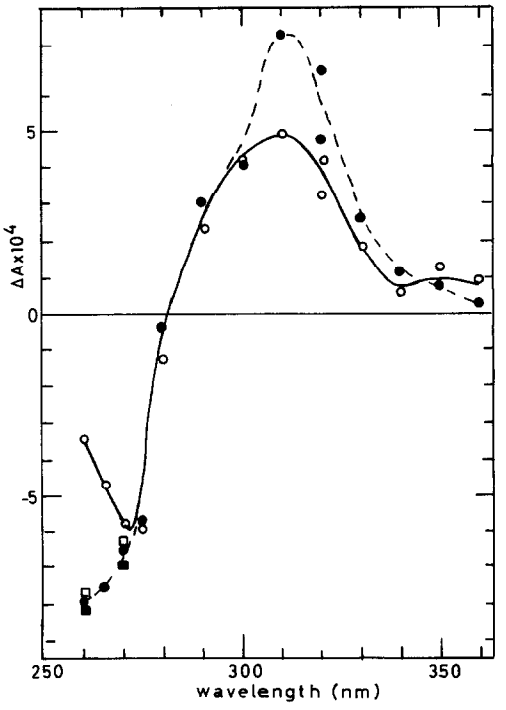

Fig. 2. Absorbance difference spectra (light *minus* dark) at −40°C. Open symbols: no additions; solid symbols: 2.5 mM ferricyanide. Below 275 nm different symbols refer to different batches of chloroplasts. Chlorophyll concentration 0.12 mM; no preillumination. Only the fast phase is plotted. The spectra are corrected for particle flattening (see ref. [13]) which varied between 1.37 at 260 nm and 1.18 at 320 nm.

Fig. 2 shows the absorbance difference spectra, in the presence and in the absence of ferricyanide. The spectra are similar to the difference spectrum obtained in vitro upon reduction of plastoquinone to its semiquinone anion [18] but in the region below 280 nm they appear to be distorted, perhaps due to interference with another compound. The contribution of this compound seems to be different in different preparations.

On basis of a differential extinction coefficient of 13 mM^{-1} cm^{-1} [18] at the maximum near 315 nm the amount of plastoquinone reduced was calculated to be 1 molecule per about 300 chlorophyll molecules, in good agreement with the number obtained by Van Gorkom [5] with detergent-treated chloroplast particles.

The spectra of fig. 2 resemble that obtained by Van Gorkom [5] at room temperature, except that the latter spectrum was shifted towards longer wavelengths, perhaps due to action of the detergent. This may also apply to the ubiquinone difference spectrum observed by Slooten [19] in bacterial reaction centers. Ultraviolet absorption difference spectra reported by Stiehl and Witt [2] and by Witt [4] showed a maximum at 325 nm, both at room temperature and at −160°C. In the region below 290 nm the first one showed much smaller absorbance changes than the spectra reported here. The reason for this discrepancy is not clear yet.

Comparison of our data with those of absorbance measurements in the green region [10,11] confirms the hypothesis [5,18] that the primary acceptor of system 2 is plastoquinone, and that its reduction to the semiquinone anion is accompanied by a band shift of C-550. Recordings d and e of fig. 1 indicate that at −40°C only 60% of the total amount of the primary acceptor is reduced with reasonable efficiency in chloroplasts preilluminated with two flashes, in agreement with similar measurements of absorbance changes near 550 nm [12] and with the results of flash experiments of Vermeglio and Mathis [10]. This supports the notion that chloroplasts converted to states S_2 and S_3 [7] by preillumination exhibit two different photoreactions at −40°C. One of these consists of an efficient photoreduction of plastoquinone, occurring in part of the reaction centers, the other one is less efficient and is correlated with the slow increase in fluorescence yield.

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