ON THE PHOTOOXIDATION OF A HIGH POTENTIAL FORM OF CYTOCHROME *b*-559

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1. Introduction

In the last few years, several reports indicated the existence of a high potential cytochrome *b*-559 in chloroplast preparations of Euglena [1] and higher plants such as lettuce, spinach or pea [2–5]. The potential of this cytochrome was found to be as high as that of cytochrome *f* (ca. + 320–370 mV), but under various conditions, such as high pH, addition of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), or antimycin A, this cytochrome is converted to a low potential form (ca. + 80 mV) [4].

Several laboratories [6–9] studying the absorption changes of cytochrome *b*-559 arrived at the conclusion that this cytochrome is located on the pathway from Photosystem 2 to Photosystem 1. Recently, it was reported by Cramer et al. [4, 5] that cytochrome *b*-559 is indeed photooxidized by Photosystem 1, but only in its low potential form. Different conclusions regarding the location of cytochrome *b*-559 have come from studies of its photooxidation at 77°K and in Tris-treated chloroplasts [10–13]. These investigators suggested that cytochrome *b*-559 is preferentially oxidized by Photosystem 2 and that it is the high potential form which is photooxidized [13]. In recent reports by Epel et al. [14, 15], a close relationship was found between C-550 and the high potential form of cytochrome *b*-559, also indicating its location near Photosystem 2.

On the basis of the experiments reported here, it is suggested that cytochrome *b*-559 which is photooxidized in the presence of an uncoupling concentration of FCCP is in its high potential form and that the photooxidation may also be mediated by Photosystem 2.

2. Methods

Chloroplasts were isolated from lettuce leaves as previously described [16]. Chlorophyll was assayed after Arnon [17]. All the measurements were carried out in an Aminco–Chance dual-wavelength spectrophotometer. The measuring beam intensity was 1–3 ergs $\times$ cm^{-2} $\times$ sec^{-1} and its half-band width 2.1 nm. Monochromatic actinic light was supplied by a 500 W projector lamp filtered through the following Schott interference filters: (a) 643 nm, half-band width of 24 nm and (b) 726 nm, half-band width of 20 nm. The intensities of the 643 nm and 726 nm actinic lights, as measured by a Yellow Spring Instrument Radiometer Model 65, were 5.6×10^4 and 6.2×10^4 ergs $\times$ cm^{-2} $\times$ sec^{-1}, respectively. Lower intensities were obtained by Schott neutral glass filters. The photomultiplier was protected from the actinic lights by a Corning 4–96 glass filter. Light absorption was measured by a home-made integrating sphere photometer.

3. Results and discussion

Fig. 1 shows the spectra obtained when chloroplast suspensions were irradiated in the presence of FCCP or the combination of FCCP and 3-(3,4-dichlorophenyl)-1,1 dimethyl urea (DCMU). In the presence of FCCP alone, both cytochrome *f* and cytochrome *b*-559 were photooxidized by 726 nm light with almost no dark reduction (upper trace). Only by irradiation with 643 nm light could photoreduction of both cytochromes be achieved (lower trace). When DCMU was introduced into the reaction medium, the spectral changes caused

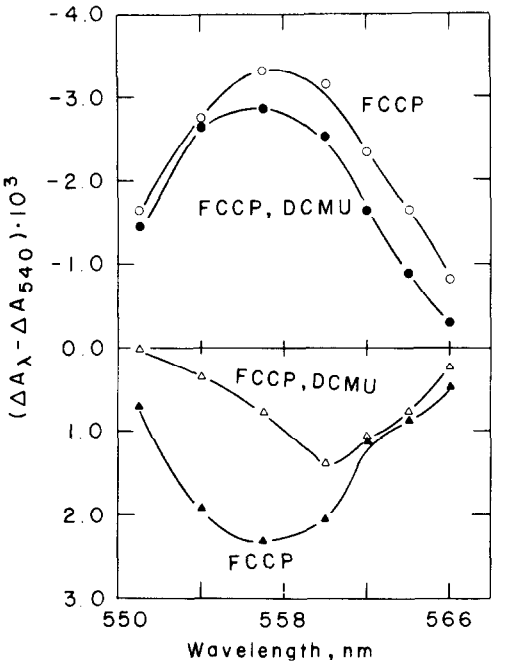

Fig. 1. Difference spectra for the photooxidation and photoreduction of cytochromes in the presence of FCCP or FCCP and DCMU. The reaction mixture contained in 0.3 ml: Tricine, pH 7.8, 60 μmoles; NaCl, 60 μmoles; $MgCl_2$, 10 μmoles and chloroplasts containing 51 μg chlorophyll/ml. FCCP (5 nmoles) with or without DCMU (10 nmoles) were introduced after 1 min illumination with 643 nm light. The suspension was then illuminated with 726 nm light for the oxidation of cytochromes (upper curves) followed by 643 nm light for their reduction (lower curves).

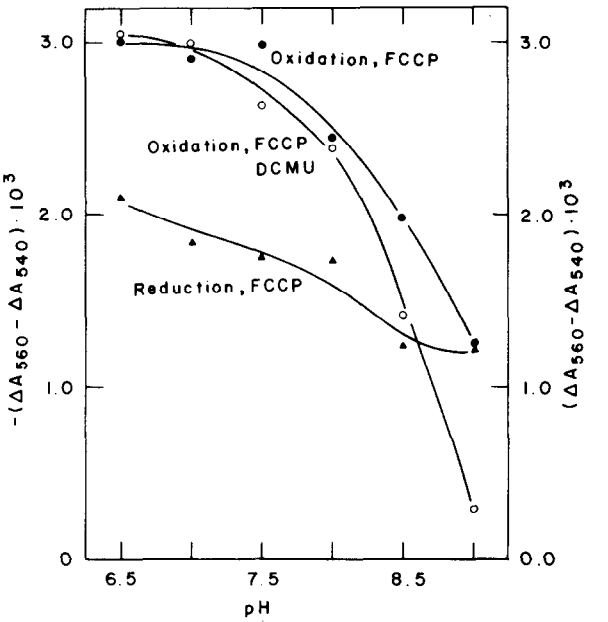

Fig. 2. The effect of pH on the photooxidation of cytochrome *b*-559. The reaction mixture as in fig. 1 with chloroplasts containing 48 μg chlorophyll/ml. The addition of 5 nmoles of FCCP or 5 nmoles FCCP plus 10 nmoles DCMU were made after 1 min illumination with 643 nm light. The extent of the photooxidation of cytochrome *b*-559 (560–540 nm) with 726 nm light was then recorded.

by irradiation with 726 nm light were rather like those with FCCP alone (upper trace), but cytochrome *f* was no longer photoreducible with 643 nm light while cytochrome *b*-559 could still be partially photoreduced (lower trace).

The pH dependence of cytochrome *b*-559 photooxidation is shown in fig. 2. In the presence of either FCCP or FCCP and DCMU the fraction of photooxidizable cytochrome *b*-559 decreased with increasing pH. This was already described for the photooxidation of cytochrome *b*-559 in absence of both FCCP and DCMU [9]. The photooxidation of cytochrome *b*-559 in presence of FCCP could be fully reversed with 643 nm light, so that a second irradiation with 726 nm light resulted in the same extent of oxidation. As seen in fig. 2 the photoreduction of cytochrome *b*-559 was rather pH independent. This is due to the difference in the degree of the dark reduction following the far-red irradiation being higher at pH 6.5 than at pH 8.0. These results are in variance with those of Cramer et al. [14], who claimed that far-red light oxidizes the low potential form of cytochrome *b*-559 and that its fraction is increased with increasing pH. Since in the experiment described in fig. 2 the chloroplasts were preilluminated to a fully reduced state of cytochrome *b*-559, it may be that the photooxidation of cytochrom *b*-559 described here is of the high potential form and that this fraction is indeed decreasing with increasing pH in accordance with Cramer's results. This assumption was shown to be true as seen in fig. 3. When cytochrome *b*-559 was photooxidized by far-red light in the presence of FCCP, almost no dark reduction followed this illumination (a), but when hydroquinone

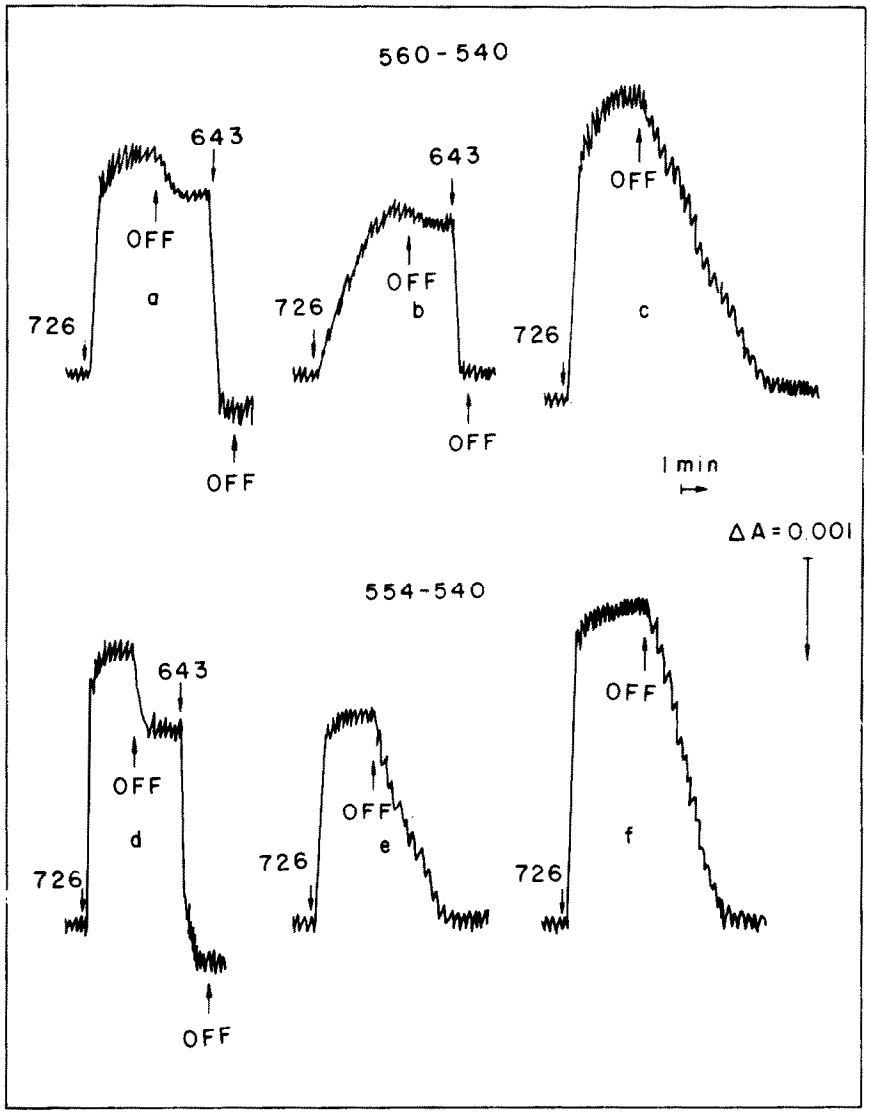

Fig. 3. The effect of hydroquinone and hydroxylamine on the photooxidation of cytochromes. The reaction mixture was as in fig. 1 with chloroplasts containing 52 μg chlorophyll/ml. The suspension was preilluminated with 643 nm light for 1 min and the additions were then made as follows: 5 nmoles FCCP to a and d, 5 nmoles FCCP plus 30 mmole, hydroxylamine pH 7.8 to b and e, and 5 nmoles FCCP plus 0.3 μmoles hydroquinone to c and f. After 3 min in the dark 726 nm light was turned on.

was introduced into the reaction medium this oxidation was fully reversed in the dark (c), and the rate of the dark reduction was no different when 1 mM ascorbate replaced the hydroquinone. When the photooxidation was carried out in the presence of hydroxylamine, the rate of oxidation was markedly slowed down, but no dark reduction could be observed (b). Since cytochrome *f* was reduced in the dark by hydroxylamine (e) and the potential of the two cytochromes is about the same, it seems that hydroxylamine does not interact directly with cytochrome *b*-559, or else, the potential of cytochrome *b*-559 is

Table 1
Quantum requirement for the photooxidation of cytochromes.

Exciting light (nm)	Cytochrome *b*-559 (quanta/electron)	Cytochrome *f* (quanta/electron)
643	12	73
726	17.3	7.5

The reaction mixture contained 60 μmoles HEPES pH 7.5, 60 μmoles NaCl, 10 μmoles $MgCl_2$ and chloroplasts containing 130 μg chlorophyll in 3.0 ml. The suspension was illuminated for 1 min with strong 643 nm light (6×10^4 ergs $\times$ cm^{-2} $\times$ sec^{-1}), then 5 nmoles FCCP and 50 nmoles of diquat were added in the dark, followed by illumination with either 643 nm light ($0.3-2.5 \times 10^3$ ergs $\times$ cm^{-2} $\times$ sec^{-1}), or 726 nm light ($1.6-2.4 \times 10^3$ ergs $\times$ cm^{-2} $\times$ sec^{-1}). The numbers given in the table are the values obtained by extrapolation to zero light intensity. Cytochrome *b*-559 and cytochrome *f* were measured at 562–540 nm and 551–540 nm respectively.

changed in its presence. It was shown by Bennoun and Joliot [18] that hydroxylamine donates electrons to Z, the donor to Photosystem 2, thus keeping Z in a fully reduced state. If one assumes that cytochrome *b*-559 and Z compete for donation of electrons to Photosystem 2, a more reduced state of Z would account for the apparent inhibition in the rate of cytochrome *b*-559 photooxidation. Such an interpretation would indicate that the photooxidation of cytochrome *b*-559 is Photosystem 2 dependent.

Finally, quantum requirement measurements were made for the photooxidation of cytochrome *b*-559 in comparison to cytochrome *f*. The results are presented in table 1. As can be seen, cytochrome *f* was indeed far better oxidized by far-red light while cytochrome *b*-559 was slightly better oxidized by short wavelength light. This again proves that cytochrome *b*-559 is at least preferentially oxidized by Photosystem 2. The high values of quantum requirement for the oxidation of cytochrome *f* by far-red light may be due to the absence of DCMU and/or the addition of FCCP.

The data reported in this paper suggest that it is the high potential form of cytochrome *b*-559 which is photooxidized in the presence of uncoupling concentration of FCCP. Quantum requirement values under low light conditions show that Photosystem 2 is at least as good in oxidizing cytochrome *b*-559 as Photosystem 1.

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