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STIMULATION OF PHOTOSYSTEM I-INDUCED OXIDATION OF CHLOROPLAST CYTOCHROME *b*-559 BY PRE-ILLUMINATION AND BY LOW pH

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SUMMARY

(1) The proportion of higher plant chloroplast cytochrome *b*-559 oxidizable during illumination by low intensity 732 nm light increases as the pH is decreased below 6.5. At pH 5.0–5.3 total oxidation is seen and subsequent red light can cause reduction of up to 2/3 of the oxidized cytochrome. The oxidation by far red light at pH 5 is inhibited by 2 μM 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone whereas the red light-induced reduction is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. In this pH range ferricyanide-oxidized cytochrome *b*-559 exists in a form not reducible by ferrocyanide.

(2) An increase in the amplitude of far-red induced oxidation also occurs at higher pH (up to pH 7.8) after pre-treatment of chloroplasts with substantially higher levels of light (approx. 10^6 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}). The degree of light activation is pH dependent, being more pronounced at lower pH. After light activation, cytochrome *b*-559 can be completely oxidized by far-red light in a manner reversible by red light up to pH values of 6, and the curve describing the amplitude of far-red oxidation as a function of pH is shifted by 0.5–1.0 pH unit toward higher pH. Far-red oxidation and red light reduction are again inhibited by 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone and 3-(3,4-dichlorophenyl)-1,1-dimethylurea, respectively.

(3) Light activation at pH 5.2–6.0 is also manifested in a small decrease in the amplitude of subsequent dark ferrocyanide reduction, and this decrease is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (10 μM).

(4) The effect of intramembranal acidity on the effective redox potential of cytochrome *b*-559 and its function is discussed.

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; PMS, *N*-methyl-phenazonium methosulphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; MES, 2-(*N*-morpholino)-ethane-sulphonic acid; MOPS, 2-*N*-(morpholino)-propane-sulfonic acid.

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INTRODUCTION

Controversy concerning the role of high potential cytochrome *b*-559 in higher plant photosynthesis stems from it being a component associated with Photosystem II [1–3], yet having a mid-point potential too high at approx. 350 mV [4–8] to participate in the main electron transport sequence. However, this mid-point describes the potential in the dark, at pH 6.5 to 8.2, and the observation of a photosystem I-mediated oxidation of cytochrome *b*-559 inhibitable by 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) [9] and *N*-methyl-phenazonium methosulphate (PMS) [10, 11] suggests that the midpoint may be functionally lower during light-induced electron transport. Observation of a decrease in ferrocyanide reducibility of cytochrome *b*-559 in chloroplasts buffered at acid pH in the dark [12] indicates that light-induced acidification of the chloroplast membrane or the *b*-559 protein itself, or generation of a transmembrane pH gradient could be the cause of a light-induced decrease in the cytochrome *b*-559 potential. In this paper further support is added to this hypothesis: Photosystem I-mediated oxidation of cytochrome *b*-559 of large amplitude in the absence of non-physiological additions to the normal reaction medium occurs at low external pH values. Moreover, turnover of cytochrome *b*-559 is increased at physiological pH values following pre-illumination of chloroplasts with high intensity light.

MATERIALS AND METHODS

Isolation of chloroplasts [13] and dual wavelength spectrophotometric measurement of cytochrome redox changes [14] were performed exactly as described previously. The room temperature difference spectra of Fig. 8 were recorded on-line to a Nova 1220 (Data General Corp.) minicomputer. The reaction medium contained 5 mM K_2HPO_4, 5 mM NaCl, 2 mM $MgCl_2$, 0.1 mM methyl viologen and additional pH buffer as indicated in the figure legends. An important difference between the incubation conditions used here at low pH and those used in ref. 12 is that the chloroplasts were not osmotically shocked before incubation in the reaction medium under the present conditions. This prevented autooxidation of cytochrome *b*-559.

RESULTS

Fig. 1 shows a Photosystem I mediated absorbance decrease at 560 nm at pH 5, where the cytochrome is not reducible by ferrocyanide (see ref. 12). The oxidation is reversed by red light, at both high intensity (Fig. 1A) and low intensity (Fig. 1B). Only relatively low intensities ($\lesssim 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}.) of far-red light mediate photooxidation, (data similar to that described previously in ref. 10 for Photosystem I-mediated oxidation in the presence of PMS). DBMIB at 2 μM inhibits the rate, and to a lesser extent the amplitude of the system I oxidation (Fig. 1C), whereas 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (5 μM) inhibits the reduction in red light (Fig. 1C and E). The relatively small effect (see Fig. 6C below) on the amplitude of the *b*-559 absorbance change is probably a consequence of use of a marginally inhibitory DBMIB concentration. Spectra for the absorbance changes of Fig. 1A are shown in Fig. 2. The spectrum for the absorbance decrease in far-red light

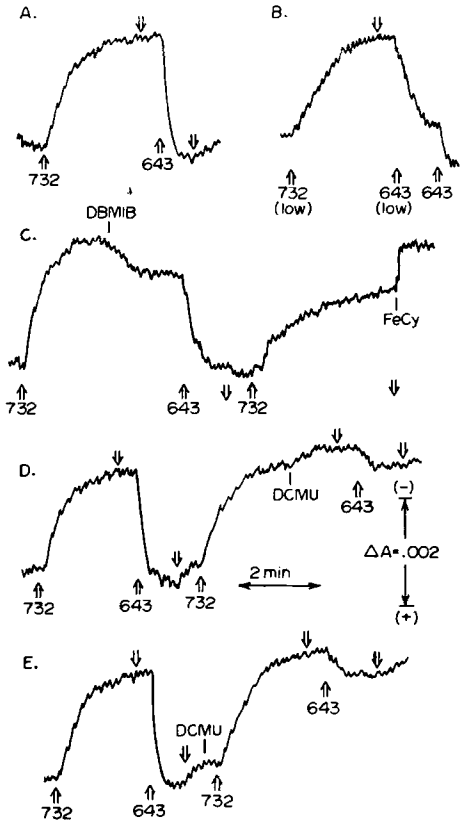

Fig. 1. Light induced absorbance changes at 560 nm at pH 5. A, Photooxidation by 732 nm light and reduction by 643 light; B, oxidation/reduction by 732 and 643 nm light of equal intensity; C inhibition of photooxidation by DBMIB; D and E, inhibition of red light reduction by DCMU; Medium buffered at pH 5 by 25 mM succinic acid plus 25 mM MES/NaOH. Light intensities (erg · $cm^{-2} \cdot s^{-1}$), 732 nm, $1.2 \cdot 10^4$; 643 nm, $6.4 \cdot 10^4$; 643 nm (low), $1 \cdot 10^4$ ergs · $cm^{-2} \cdot s^{-1}$. DBMIB, 2 μM; DCMU, 5 μM; ferricyanide, 250 μM; chlorophyll, 80 μg/ml; reference wavelength, 570 nm.

shows a maximum at 558–559 nm with a strong shoulder at 55 nm, indicating significant oxidation of both cytochromes *f* and *b*-559. Addition of ferricyanide (250 μM) immediately after far-red oxidation in fact gives no further absorbance decrease. The maximum of the absorbance increase in red light is shifted slightly by 1–2 nm to longer wavelengths, a reflection either of incomplete cytochrome *f* reduction in coupled chloroplasts or possibly photoreduction of cytochrome b_6 [15]. Also shown is an ascorbate minus ferricyanide spectrum recorded at the end of the experiment to normalize the light-induced absorbance changes; approximately 2/3 of the total cytochrome *b*-559 is observed to be oxidized by far-red light and reduced by red light at low pH.

Figs. 3 and 4 (closed circles) show that there is a systematic increase in the amplitude of far-red induced cytochrome *b*-559 oxidation as the pH of the medium is decreased from 6.5 to 5.0, with an effective pK, or half-amplitude pH, for this event approximately equal to 5.3–5.8. There is an approximately parallel decrease in the

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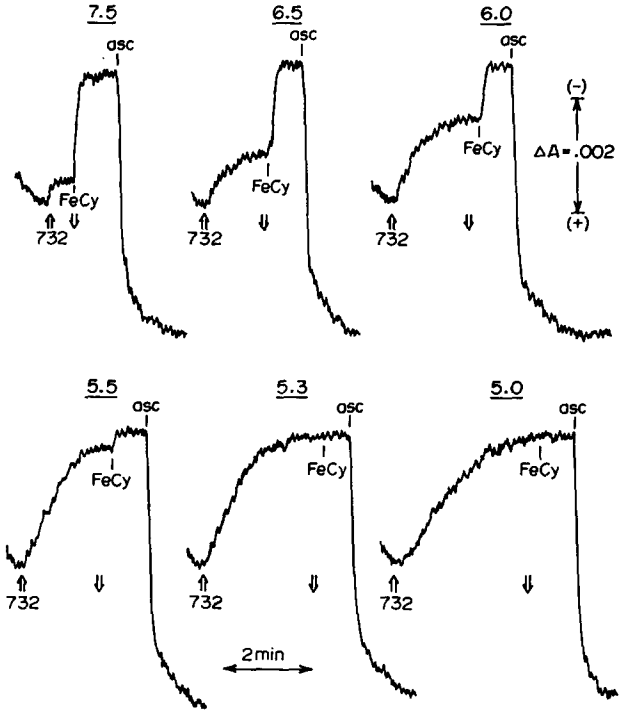

Fig. 3. Photooxidation of cytochrome *b*-559 by 732 nm light at different pH values. 732 nm light intensity, $1.2 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. pH 5 to 5.5 buffered by 25 mM succinic acid and 25 mM MES; pH 6.0 and 6.5 by 5.0 mM MES; pH 7.5 by 50 mM Tricine. All pH values adjusted with NaOH. Ferricyanide, 250 μM; ascorbate, 2 mM; chlorophyll, 80 μg/ml; reference wavelength, 570 nm.

experiments were not osmotically shocked prior to incubation in the reaction medium. This prevented the autooxidation previously observed in ref. 12 upon incubation at low pH, although there is similar decrease in ferrocyanide reduction at low pH in both kinds of chloroplast preparations (crosses in Fig. 4).

A role of high intensity light in modifying the pH dependence of this photooxidation can also be defined. The participation of a light-activated process in stimulation of *b*-559 oxidation by Photosystem I is shown in Figs. 4 and 5. If chloroplasts are first pre-treated with 1–2 min of high intensity 643 nm light and the degree of cytochrome *b*-559 oxidation in subsequent low intensity far-red light recorded, complete oxidation can now be demonstrated at higher pH, up to pH 6 (Fig. 4). Light activation at pH 5.5 is shown in Fig. 5. Note here that the amplitude of far-red light induced oxidation in Figs. 5B and C is increased by a reduction of cytochrome *b*-559 occurring during the high intensity red light treatment, but the far-red oxidation is in any case more complete as judged by the effect of added ferricyanide. Successive cycles of high intensity red light and low intensity far-red light cause the turnover of virtually the whole pool of cytochrome *b*-559 (Fig. 5).

It is seen that light activation at pH 7.5–8.0 with the intensities used in Fig. 4 is negligible. The intensity of 643 nm light ($6 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}) is, however,

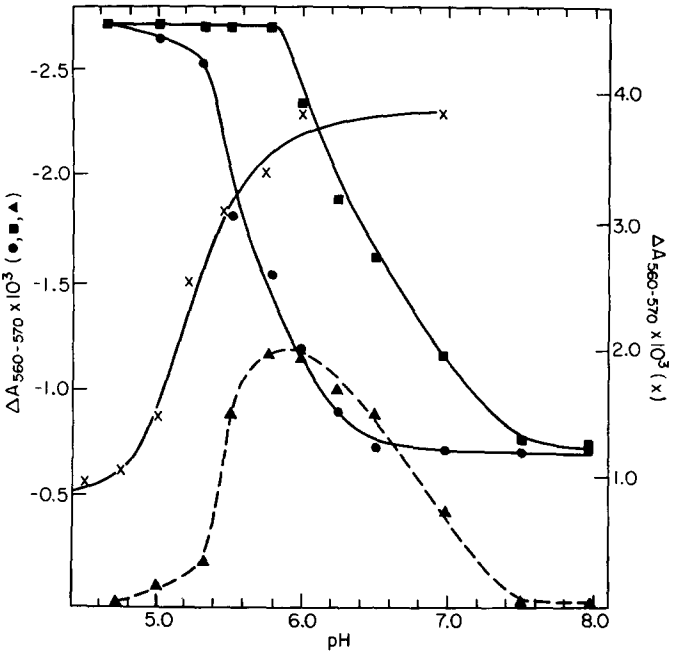

Fig. 4. Dependence on pH of the photooxidation of cytochrome *b*-559 observed (●) before pre-illumination; (■) after pre-illumination with 1 min of 643 nm light, intensity $6.4 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}; (▲) and dashed curve, calculated absorbance change due to light activation. Conditions as in Fig. 3. pH 7 buffered with 50 mM MOPS. Ferrocyanide (2 mM) reduction of *b*-559 chemically oxidized by ferricyanide (0.25 mM) is shown in curve with crosses (×).

still a fairly low intensity compared to those used to saturate total photosynthesis, particularly at the high chlorophyll concentrations ($\approx$ 80 μg/ml) used here. Fig. 6A shows that pre-illumination with higher intensity red light (defined by a Corning CS 2-62 cut-off filter with 2 cm of $CuSO_4$ solution as a heat filter, intensity $\approx 10^6$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}) can induce a small stimulation of a slow far-red oxidative change at 560 nm at pH 7.8. After far-red illumination, about 40 % of the cytochrome is observed in an oxidized state (compare to Fig. 6B). The photooxidation is inhibited by 3 μM DBMIB, in a manner partially reversible by subsequent addition of 20 μM benzoquinone (Fig. 6C), whereas DCMU tends to slightly stimulate photooxidation, and to completely inhibit reduction by Photosystem II (Fig. 6D). The light activation was not due to heating effects; no temperature change in the sample could be recorded during the two min illumination period and increasing the temperature to 30 °C for short times had no effect on cytochrome *b*-559 photooxidation. Photooxidation again depends on the use of low intensity (10^3–10^4 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}) far-red light (Fig. 7), clearly demonstrating the requirement for Photosystem I excitation.

Spectra for the absorbance changes after light activation at pH 7.8 are shown in Fig. 8. Fig. 8A shows the total chemical change (ferricyanide minus dark) due to complete cytochrome oxidation by ferricyanide. The control (far-red minus dark) spectrum indicates predominantly cytochrome *f* oxidation (Fig. 8B) and the difference A–B (Fig. 8C) shows that cytochrome *b*-559 stays almost entirely reduced as usual

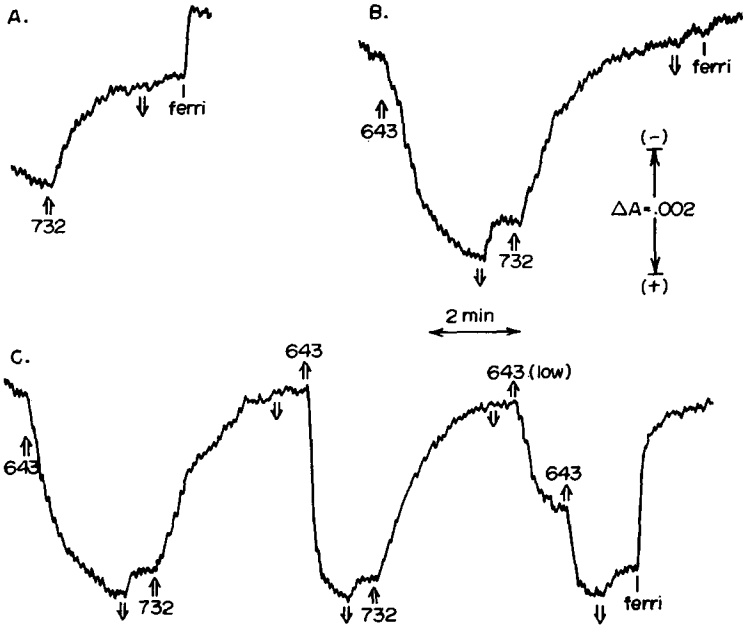

Fig. 5. Effect of pre-illumination with red light on absorbance changes at 560 nm at pH 5.5. A, photooxidation before pre-illumination; B, photooxidation and ferricyanide addition after far-red light to show extent of photoooxidation; C, photooxidation and reduction following pre-illumination. Light intensities (erg · cm^{-2} · s^{-1}): 732 nm, 0.8 · 10^4; 643 nm (low), 1.1 · 10^4; 643 nm, 1.1 · 10^5. Ferricyanide, 250 μM; ferrocyanide, 2 mM; ascorbate, 2 mM; chlorophyll, 80 μg/ml. pH buffered at 5.5 with 25 mM succinic acid plus 25 mM MES. Reference wavelength, 570 nm.

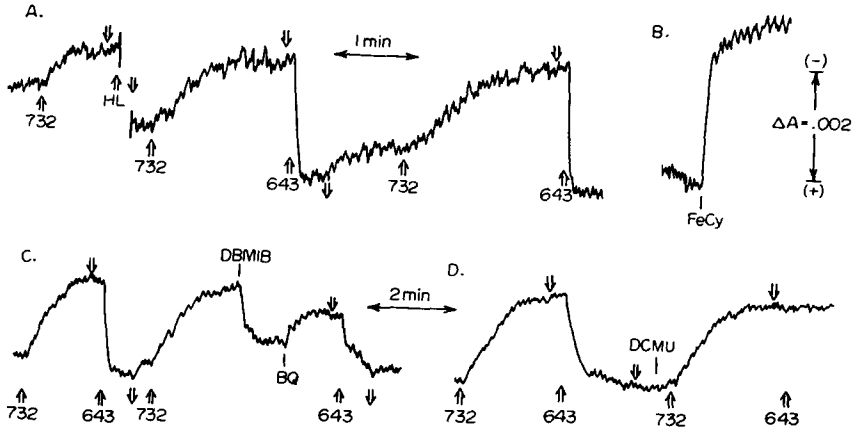

Fig. 6. Stimulation of light induced absorbance changes at pH 7–8 by pre-illumination. A, photooxidation before and after pre-illumination with high light (HL); B, complete oxidation by ferricyanide (250 μM); C, inhibition of photooxidation by DBMIB (3 μM) and partial restoration by benzoquinone (20 μM); D, inhibition of 643 induced reduction by DCMU (5 μM). pH 7.8 in A–C, pH 7 in D. High light defined by Corning CS 2-62 red filter plus 2 cm 1 % $CuSO_4$ solution, giving an intensity of approx. 10^6 ergs · cm^{-2} · s^{-1}. 732 nm light intensities (erg · cm^{-2} · s^{-1}); 2.4 · 10^3 in A and B, 3.8 · 10^3 in D. 643 nm, 1.6 · 10^4 erg · cm^{-2} · s^{-1}. Chlorophyll, 75 μg/ml. Reference wavelength, 570 nm.

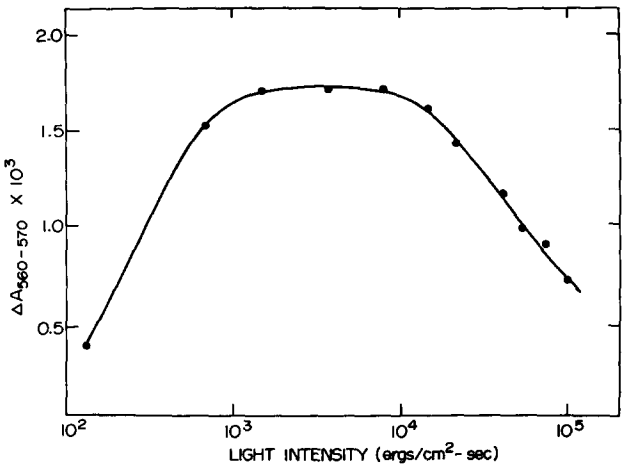

Fig. 7. Dependence of photooxidation at 560 nm after pre-illumination on the intensity of 732 nm light. Pre-illumination with high red light as defined in Fig. 6. Reaction pH buffered at pH 7. 732 nm intensity adjusted by suitable combinations of neutral density filters and measured using a Yellow Springs Radiometer. Reference wavelength, 570 nm. Chlorophyll, 75 μg/ml.

under these illumination conditions employing moderate intensity far-red light. However, when the same conditions of far-red actinic illumination are used to test *b*-559 oxidation after the sample has been preilluminated with higher intensity ($\approx 10^6$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}), the peak of the far-red minus dark spectrum is shifted to 556 nm (Fig. 8D), showing that there has been a significant increase in *b*-559 photooxidation by far-red light after preillumination.

Further demonstration of a significant amplitude of *b*-559 photooxidation after preillumination is provided by a comparison of Fig. 8E (A–D) versus Fig. 8A, and Fig. 8F (far-red after preillumination minus far-red, peak at 560 nm) versus Fig. 8B (far-red minus dark, peak at 554 nm). Fig. 8G (Dark after preillumination minus dark without preillumination) shows that the preillumination regime itself caused little photooxidation of *b*-559.

The inhibition by DBMIB of cytochrome *b*-559 oxidation by Photosystem I light (Fig. 6C) suggests that light activation induces the formation of low potential *b*-559, but Figs. 9A and B show that illumination with 2 min of high intensity red light at pH 7 does not influence the subsequent ability of ferrocyanide to reduce cytochrome *b*-559. However, at lower pH values, between 5.25 and 6.00, a small but consistent decrease in the amplitude of reduction with ferrocyanide occurs after light activation with high intensity red light (Figs. 9C and D). The difference in ferrocyanide reduction of ferricyanide-oxidized cytochrome *b*-559 at pH 7 (Fig. 9B) and 5.5 (Fig. 9D) is again observed. The decrease in ferrocyanide reducibility due to pretreatment with red light at pH 5.5 is inhibited by 10 μM DCMU (Fig. 9E). In fact, after pre-illumination at low pH in the presence of DCMU, amplitudes of cytochrome *b*-559 reduction by ferrocyanide are consistently greater. Table I summarizes data taken from a series of traces like those shown in Fig. 9. The pH dependence for

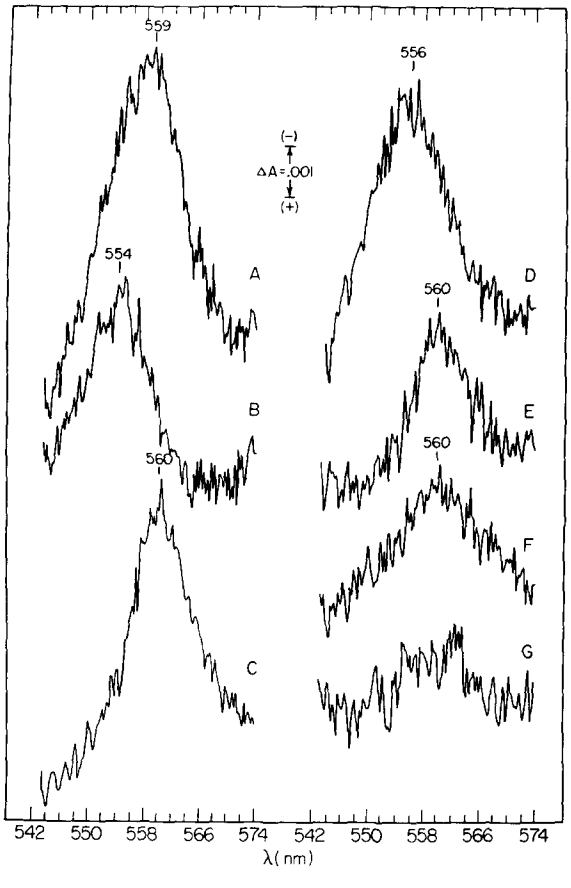

Fig. 8. Room temperature difference spectra recorded during a light-activation experiment at pH 7.8. A, total oxidizable cytochrome *f* and *b*-559 content defined by ferricyanide minus dark difference; B control far-red minus dark spectrum; C, A minus B, ferricyanide minus far-red oxidized; D, (far-red after preillumination) minus (dark after pre-illumination); E, A minus D, (ferricyanide) minus (far-red after pre-illumination); F, D minus B or C minus E, (far-red after preillumination) minus (far-red); G, (dark after pre-illumination) minus (dark). Individual spectra were recorded on-line to a digital computer. Sums of 4 spectra acquired from different experimental runs were used to compute the difference spectra. 732 nm intensity, $2.4 \cdot 10^3$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Pre-illumination as in Fig. 6. Ferricyanide, 500 μM. Chlorophyll, 100 μg/ml.

the amplitude of the absorbance increase at 560 nm caused by ferrocyanide addition before and after light activation is shown in Fig. 10. Difference spectra have shown that reductive changes measured within 0.5 min after ferrocyanide addition are due mostly to cytochrome *b*-559, perhaps due to the excess of *b*-559 over *f*, and also because of the inaccessibility of cytochrome *f* to the highly charged reductant [12, 14].

The presence of the uncouplers NH_4Cl (10 mM) or gramicidin (10 μg/ml) has no effect on the light activation phenomenon shown in Fig. 9 or on the far-red light induced oxidation at low pH.

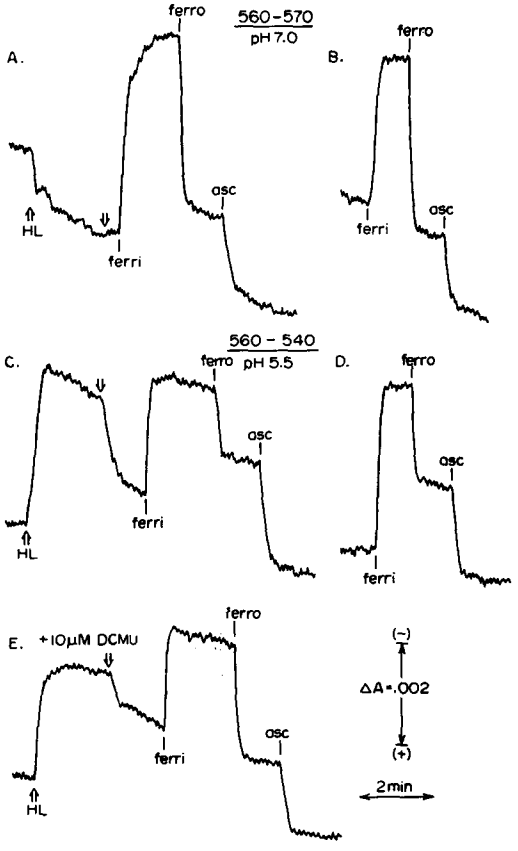

Fig. 9. The effect of pre-illumination with high intensity red light on the ability of ferrocyanide to reduce cytochrome *b*-559. A, After preillumination at pH 7.0; B, 7.0, control; C, After pre-illumination at pH 5.5; D, pH 5.5, control; E, After pre-illumination at pH 5.5 in the presence of 10 μM DCMU. High intensity light (HL) defined as in Fig. 6. Reference wavelength: 570 nm in A and B; 540 nm in C, D and E. pH 7 buffered by 50 mM MOPS, pH 5.5 by 25 mM succinic acid plus 25 mM MES. Ferricyanide, 250 μM; ferrocyanide, 2 mM; ascorbate, 4 mM; chlorophyll 80 μg/ml.

TABLE I

EFFECT OF PRE-ILLUMINATION AND DCMU ON THE EXTENT OF REDUCIBILITY OF CYTOCHROME *b*-559 BY FERROCYANIDE AT pH 5.5.

Conditions were as described in the legend to Fig. 9. Experiment I used the wavelength pair 560–540 nm and II, 560–570 nm. *n* is the number of experimental trials.

Conditions	Amplitude of reduction by ferrocyanide ($\Delta A \cdot 10^4$)	
	I	II
Dark	18 ± 1 ($n = 5$)	29 ± 1 ($n = 6$)
After pre-illumination	12 ± 1 ($n = 3$)	22 ± 1 ($n = 6$)
After pre-illumination Plus DCMU	23 ± 1 ($n = 5$)	33 ± 1 ($n = 2$)

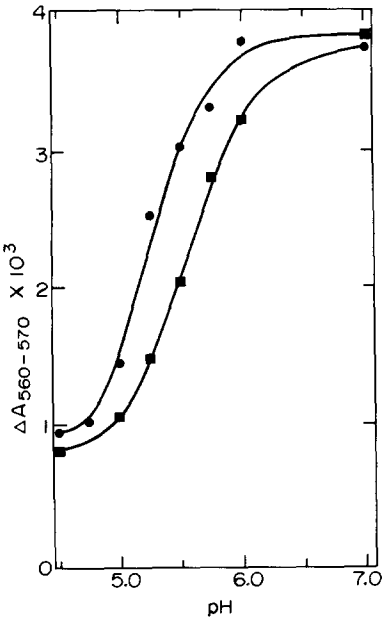

Fig. 10. pH dependence for the decrease in amplitude of ferrocyanide reducibility of cytochrome *b*-559 caused by pre-illumination with high intensity light. (●) before pre-illumination; (■) after pre-illumination. Conditions as in Fig. 9. pH 4.5 and 4.75 buffered by 50 mM succinic acid. Other pH's were buffered as described under Fig. 4. Chlorophyll, 80 μg/ml.

DISCUSSION

(*1*) *Photooxidation of cytochrome b-559 at low pH*

Cytochrome *b*-559 in the chloroplast preparations incubated at low pH as described has a negatively shifted redox potential in that it loses ferrocyanide reducibility. We do not yet know the exact extent of this potential change but estimate it to be at least 50–100 mV since the midpoint of the cytochrome is approx. +350 mV and an excess of ferrocyanide establishes a redox potential of approximately+300–350 mV under our experimental conditions. This modified high potential *b*-559 remains reduced for an appreciable time in the dark, but can be reversibly photooxidized by system I through a pathway inhibited by low concentrations of DBMIB. As the pH is decreased toward a value of 5, the amount of cytochrome *b*-559 undergoing such photooxidation approaches the total amount of *b*-559 present in the chloroplasts. This data might suggest that at low pH a lower potential state (or states) of cytochrome *b*-559 is created which can equilibrate thermodynamically with the plastoquinone pool and contribute electrons to Photosystem I. A role for low inter- or intramembranal pH in facilitating the *b*-559 electron donation to system I is further implied by the ability of strong preilluminating light, which causes light-induced proton uptake, to stimulate the amplitude of the subsequent oxidation by far-red light.

It is proposed that during the preillumination with high light intensity the local proton concentration around cytochrome *b*-559 is increased, again creating a

low potential *b*-559 capable of being oxidized by plastoquinone. After the high light is turned off the system rapidly relaxes, as protons efflux from the thylakoid. However, sufficient protons, perhaps bound to fixed negative charges exposed during illumination, could remain so as to lower the threshold for the potential shift in subsequent low intensity far-red light. That a shift in potential does occur after illumination with strong light is suggested by the observation of a residual change in ferrocyanide reducibility approximately 20 s after pre-illumination at lower pH (Figs. 9 and 10).

In connection with the thermodynamic problems of electron or hydrogen transfer from high potential *b*-559 to plastoquinone it should be noted also that the midpoint potential of plastoquinone should be shifted positively by decreasing pH ($\Delta E_m/\Delta pH = -59$ mV at 25 °C for quinone). Thus, if $E_{m7} \approx 110$ mV [19], $E_{m5} \approx +230$ mV. A major question, however, about the significance of a pathway from cytochrome *b*-559 to Photosystem I is the slow rate of the Photosystem I-mediated oxidation of *b*-559. The characteristic half-times for initial rates of photooxidation under the conditions employed are at best 5–10 s and often 30–60 s. However, these conditions involve the use of intensities ($\approx 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}) of 732 nm light which are very far from saturating Photosystem I. In addition, electron transport between the two photosystems is strongly inhibited at low pH. The explanation of the slow photooxidation rates of *b*-559 may then be linked to the problem of why the amplitude of the Photosystem I-mediated oxidation saturates at such low light intensities. The most probable explanation for this saturation of the far-red light-induced absorbance decrease at 560–561 nm resides in interference from the positive absorbance change peaking at 563 nm arising from photoreduction of cytochrome b_6. An incident far-red light intensity of 10^4 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} of 732 nm light would not allow observation of a fast oxidation of *b*-559 both because of a small rate of absorbed quanta and, extrapolating from the pH and light activation experiments, because of a low rate of proton pumping into or across the thylakoid membrane.

(2) The site of action of low pH on cytochrome b-559

The ability of non-permeant acids which will not potentiate acid-base phosphorylation to cause (a) the reversible dark acid-induced oxidation of cytochrome *b*-559 [12] and (b) a midpoint potential decrease in the experiments of ref. 12, and those reported here, implies that the role of the acid is not to create a pool of internal acidity or a pH gradient. The insensitivity to phosphorylation uncouplers of the effects of low pH on cytochrome *b*-559 also implies that acidification of the membrane or the *b*-559 protein itself is involved rather than a pH gradient. The observation of a high spin ferric heme electron spin resonance signal whose integrated intensity shows a pH dependence very similar to that of the acid-induced oxidation of *b*-559 [20] suggests at this time that the acid effect is directed at a site close to the *b*-559 heme presumably involving the *b*-559 protein. As to whether the protonation site is closer to the inside or the aqueous surface of the membrane, the approximate mid-pH value of ≈ 5.5 at which cytochrome *b*-559 undergoes a change in properties does match that estimated for the inner thylakoid space [21, 22], and is quite distinct from the mid-pH value of ≈ 7.5 for a surface conformational change such as reactivity of coupling factor with *N*-ethyl-maleimide. [23]. The heme of high potential *b*-559 is, however, relatively close to the membrane surface in terms of accessibility to the charged oxidant ferricyanide [14], although the protein moiety of *b*-559 has a molecular

weight of 46 000 [24] and the site of protonation need not be immediately adjacent to the heme. The conditions for the dark acid-base induced redox changes of *b*-559 reported in ref. 12 are similar to those used to observe a reversible acid-base induced changes in chloroplast fluorescence yield [25].

The site of protonation of cytochrome *b*-559 relative to the inside and outside of the membrane bears on the question of a physiological source for protons affecting *b*-559 during steady-state photosynthesis. The general source of such protons could be the external aqueous medium or the intramembranal water involved in O_2 evolution.

(3) Site of action of DCMU

If the mechanism of the decrease in ferrocyanide reducibility caused by pre-illumination with high actinic light intensities involves protonation of the cytochrome *b*-559 protein, the ability of DCMU to prevent this potential change and to stabilize the high potential ferrocyanide-reducible form of cytochrome *b*-559 suggests that one site of action of DCMU is cytochrome *b*-559. This conclusion is supported by inhibition of the dark acid-induced decrease in ferrocyanide reducibility by low concentrations of DCMU [26].

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