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High and Low Potential States of the Chloroplast Cytochrome *b*-559 and Thermodynamic Control of Non-cyclic Electron Transport

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Abstract

Most of the chloroplast *b*-559 is high potential at neutral pH as defined by hydroquinone reducibility. FCCP* (20 μM) and antimycin A (50 μM) convert high potential *b*-559 to a low potential state which can be reduced by ascorbate but not hydroquinone. The low and high potential states of cytochrome *b*-559 are different forms of the same cytochrome.

Three lines of evidence indicate that the cyt *b*-559 oxidized by photosystem I is low potential: (1) the *b*-559 photooxidized by far-red light in the presence of FCCP (3 μM) is low potential *b*-559; (2) the amplitude of the *b*-559 oxidation by far-red light and the amount of low potential *b*-559 present in the dark have the same general dependence on pH; (3) inhibitor studies show that plastoquinone mediates the oxidation of cyt *b*-559 by PS I.

The well-known stimulation of *b*-559 oxidation by far-red light in the presence of FCCP is attributed to FCCP-facilitated photoconversion of high potential *b*-559 to a low potential form.

It is concluded that if cyt *b*-559 is oxidized by system I light, then it is a low potential form ($E_{m7} \approx +80$ mV) which is oxidized. It is not proven, however, that a significant amount of cyt *b*-559 is oxidized by PS I under coupled or physiological conditions.

Possible thermodynamic regulation of non-cyclic electron flow involving the distribution between high and low potential forms of cyt *b*-559 is discussed.

Introduction

The oxidation-reduction potentials of cyt *b*-559 and *b*-563 in spinach chloroplasts have been measured recently by Fan and Cramer[1] under anaerobic conditions designed for accurate measurements at low potentials. The midpoint potential of cyt *b*-559 was found to be $+80 \pm 20$ mV at pH 7·0. Nakatani and Hind[2] have obtained a similar potential value ($E_{m7} = +55$ mV) for cyt *b*-559 in spinach chloroplasts treated with triton-X-100[2]. Ikegami *et al.* have observed a high potential *b*-558 in *Euglena* chloroplasts and have also shown that the pyridine hemochrome is *b*-type.[3] Knaff and Arnon have titrated a

* Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxy phenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; cyt, cytochrome; PS, photosystem; E_{m7}, midpoint potential at pH 7·0; mV, millivolt.

b-559 of high potential in spinach chloroplasts.[4] It has been reported by Bendall[5] that pea chloroplasts have both a high and a low potential form of a 559 nm cytochrome differing in potential by about 350 mV. The midpoint of the high potential *b*-559 in *Euglena*, spinach, and pea chloroplasts is approximately +350 mV.[3–5].

Though it has been recognized that the light-induced reactions of cyt *b*-559 are more complicated than those of cyt *f*, room temperature studies of the cyt *b*-559 photoreactions in *C. reinhardi* chloroplast fragments,[6] and chloroplast fragments from spinach[7, 8] and lettuce[9] have concluded that cyt *b*-559 is on the pathway from PS II to PS I. Thermodynamic studies and analogy with mitochondria suggest that cyt *b*-559 functioning efficiently in this pathway should have a low potential ($E_{m7} \leqslant 100$ mV).[1]

Different conclusions regarding the position of cyt *b*-559 relative to the two photosystems have come from studies of the *b*-559 photoreactions at 77° K and in Tris-treated chloroplasts. Photosystem II light preferentially oxidizes cyt *b*-559 at 77° K,[10–13] and it is high potential cyt *b*-559 which is involved in this reaction.[13] This suggests that cyt *b*-559 may be on a side pathway connected to the reaction center of PS II,[11–13] or between two light reactions of PS II.[4]

The anomalous behavior of cyt *b* in chloroplasts is similar to that which has been observed in mammalian mitochondria, and the controversy over whether the cyt *b* is in or out of the main electron transport chain has its precedent in the mitochondrial studies.[14] A recent suggestion is that there is a cyt *b* component in pigeon heart and rat liver mitochondria which undergoes energy-dependent changes in redox potential involved in the regulation of respiratory electron transport.[15, 16]

On the basis of the experiments reported here, we have concluded that the cyt *b*-559 in chloroplasts has both low and high potential forms which can be interconverted. In this work we define the high potential *b*-559 by its being hydroquinone reducible. The low potential cyt *b*-559 is defined here by its being ascorbate reducible, but not hydroquinone reducible. The cyt *b*-559 which is oxidized by PS I is low potential *b*-559. It is suggested that the rate of non-cyclic electron transport may be partly regulated by the distribution between low and high potential states of cyt *b*-559.

Methods

Spinach chloroplasts were prepared as described previously[1, 17] and lettuce chloroplasts by the method of Neumann *et al.*[18] Fresh preparations were coupled for at least 3 hours, as judged by ADP stimulation of O_2 evolution with ferricyanide as acceptor by a factor of 1·4–1·7. The chloroplasts used in Figs. 2 and 3 had been stored in liquid nitrogen. Cytochrome absorbance measurements were made in an Aminco-Chance dual wavelength spectrophotometer using a PAR lock-in amplifier as previously described.[1, 17] The measuring beam intensity was about 1 erg/cm^2-sec with a half band-width of 2·1 nm. Actinic light intensities (in ergs/cm^2-sec incident on the cuvette) are $6{\cdot}3 \times 10^4$ and $7{\cdot}0 \times 10^4$ at 645 and 713 nm, respectively, for the cytochrome absorbance measurements and 3×10^5 of white light filtered by 5 cm of 1% $CuSO_4$ solution for O_2 evolution. O_2 was measured with a YSI model 53 amplifier and bath assembly using a model 5331 sensor. 0·7 mM ferricyanide was used as electron acceptor. Antimycin A and FCCP were obtained, respectively, from the Sigma Chemical Company and Dr. P. G. Heytler. We thank Professor A. Trebst for a gift of DBMIB.

Results

Hydroquinone (10^{-4} M) will reverse most of the ferricyanide induced oxidative absorbance change of the cyt *b*-559 in spinach chloroplasts at pH 7·0 (Fig. 1). The measurement is made at 562 nm to minimize the contribution of cyt *f*. Initially the cyt *f* and *b*-559 are reduced and the *b*-563 is oxidized in the dark[7] so that the latter does not contribute at all to the oxidative absorbance changes. As the midpoint potential of the benzoquinone–hydroquinone couple is approximately +0·26 volts at pH 7·0 and 25°,[19] this experiment indicates that at pH 7·0 in the dark most of the cyt *b*-559 has a potential much more positive than that measured by Fan and Cramer.[1] This can partly be attributed to

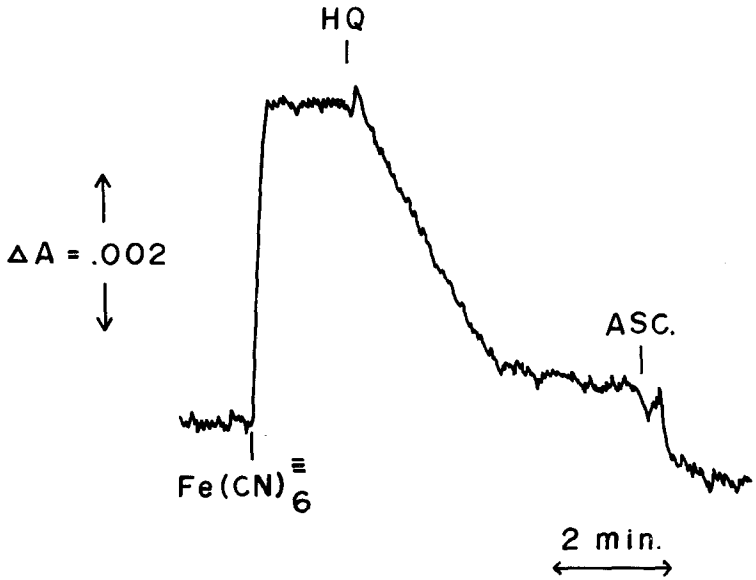
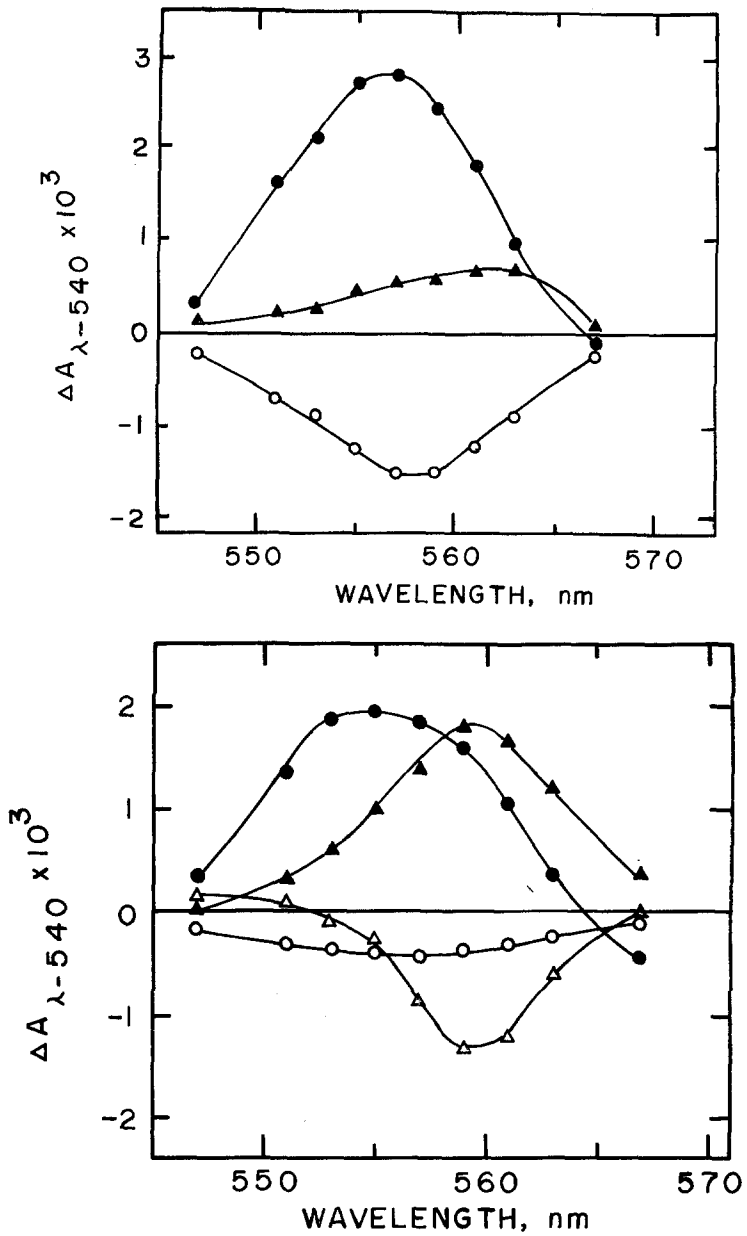


Figure 1. Dark oxidation and reduction of cyt *b*-559 in spinach chloroplasts. Oxidant, 60 μM ferricyanide; reductants, 10^{-4} M hydroquinone and 10^{-3} M ascorbate. Reaction medium: 20 mM MOPS, 10 mM NaCl, 5 mM phosphate, pH 7·0. 90 μg/ml chlorophyll. Cyt *b*-559 measured at 562 nm with 540 nm as reference with upward deflection representing a decrease in absorbance at 562 nm relative to 540 nm.

chloroplast ageing and uncoupling in the times needed to perform the redox titrations of ref. 1.

Difference spectra for the cytochrome oxidation by ferricyanide and successive reduction by hydroquinone and ascorbate in lettuce chloroplasts at pH 6·8 are shown in Fig. 2a. The peak of the spectrum for ferricyanide oxidation is at 558 nm, reflecting the oxidation of more *b*-559 than *f*. The peak for the subsequent hydroquinone reduction is at 557 nm. Lettuce and spinach chloroplasts give similar results in these dark experiments except that part of the cyt *b*-559 appears to be oxidized initially in lettuce chloroplasts (Fig. 2 and ref. 9).

It has been pointed out by Plesničar and Bendall[20] that the amount of hydroquinone-reducible cyt 559 is decreased by triton-X-100. In addition, among chemical agents known to affect chloroplast electron transport, FCCP and antimycin A will increase



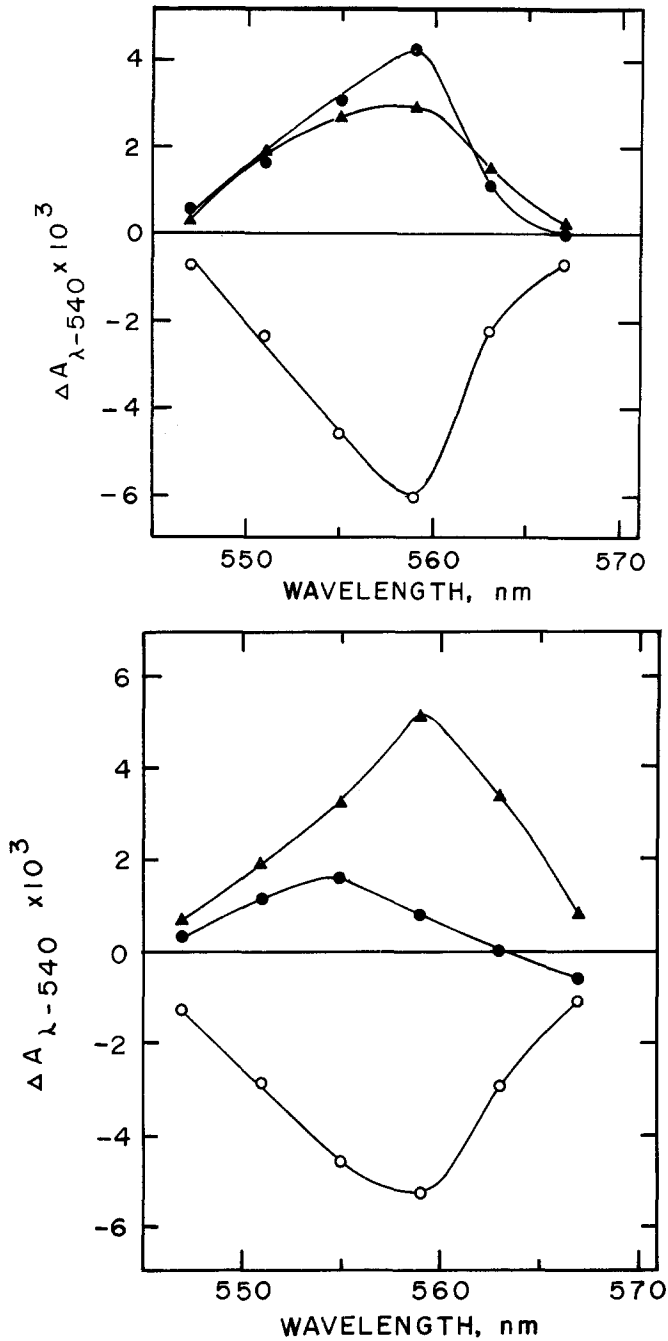
Figures 2a,b: Difference specta for dark oxidation and subsequent reduction of cyt *f* and cyt *b*-559 in lettuce chloroplasts, control (a) and with 20 μM *p*-CF_3O–CCP (b). (a) Lettuce chloroplasts which had been stored in liquid nitrogen were suspended at 100 μg/ml chlorophyll in 20 mM P_i, 20 mM NaCl, 4 mM $MgCl_2$ at pH 6·8. (○), oxidation upon addition of 60 μM ferricyanide; (●), reduction upon subsequent addition of 0·2 mM hydroquinone; (▲), further reduction upon addition of 1 mM ascorbate. (b) Chloroplasts resuspended as in (a) but incubated with 20 μM *p*-CF_3O–CCP for 5 minutes. (△) Oxidation by *p*-CF_3O–CCP; (○) further oxidation by addition of 60 μM ferricyanide; (●), reduction by addition of 0·1 mM hydroquinone; (▲), further reduction by third addition of 1 mM ascorbate. Reference wavelength, 540 nm.·

the amount of low potential *b*-559 if incubated in the dark at concentrations somewhat higher than needed to affect light-induced electron transport (Figs. 2b, 3a, b). A 5 minute dark incubation with 20 μM FCCP causes the oxidation of most of the *b*-559 which is not initially oxidized in lettuce chloroplasts (Fig. 2b). There is little further oxidation by ferricyanide. The peak of the subsequent reduction by hydroquinone is at 555 nm, and the peak for further reduction by ascorbate is at 559 nm. The decrease in the amplitude of the hydroquinone reduction at 559 nm after incubation with FCCP is about 0·001 absorbance units and this is accompanied by an approximately equal increase in the ascorbate reduction at 559 nm. Figure 3a shows difference spectra for cytochrome oxidation by ferricyanide and successive reduction by ferrocyanide and ascorbate at pH 7·8 in spinach chloroplasts. More than half the cyt *b*-559 is reducible by ferrocyanide. The E_{m7} of ferri-ferrocyanide is about +0·43 volts.[19] Upon treatment with antimycin A (50 μM) for 5 minutes in the dark, the peak of the spectrum for ferrocyanide reduction shifts from 559 nm (Fig. 3a) to 555 nm (Fig. 3b). After antimycin treatment all of the cyt *b*-559 is ascorbate reducible though not ferrocyanide reducible. The antimycin A did not cause any cyt *b*-559 oxidation, only an apparent change of potential. Within the accuracy of the experiment there does not appear to be an antimycin-induced red shift in peak wavelength of the ascorbate-reducible *b*-559, as has been reported in mammalian mitochondria[21] and anaerobic yeast mitochondria.[22] Effects of FCCP and antimycin A on mitochondrial cyt *b* similar to those shown in Figs. 2 and 3 have been reported in ref. 22. The potential modifying effects of FCCP and antimycin A shown above may be due to a direct interaction with cyt *b*-559 or to a membrane interaction which modifies the environment of the *b*-559. The oxidation caused by the FCCP is presumably due to the latter.

Since the decrease in the amount of high potential *b*-559 is accompanied by an approximately equal increase in the amount of low potential *b*-559, it is concluded that high and low potential *b*-559 represent different forms of the same cytochrome.

The fraction of the ferricyanide oxidized *b*-559 which is high potential and hydroquinone-reducible is a decreasing function of pH, unlike the cyt *f*, whose hydroquinone reducibility is pH-independent (Fig. 4). The cyt *f* and *b*-559 measurements are made at 551 and 562 nm, respectively, so as to minimize the spectral overlap of the two. Reduction is measured with successive additions of hydroquinone and ascorbate as in Fig. 1. Hydroquinone becomes increasingly unstable as the pH is raised and the experiments cannot be carried out above pH 8·4. Below pH 8·4 the hydroquinone is stable for a longer time than required for reduction, and the pH-independence of the cyt *f* reduction serves as a control for the cyt *b*-559. Thus, at pH 8·0 where the midpoint potential of the benzoquinone–hydroquinone couple would be +0·20 volts, only about half of the oxidized *b*-559 is high potential as defined by hydroquinone reducibility. The fraction of the *b*-559 which is ascorbate, but not hydroquinone-reducible, we tentatively identify with the low potential ($E_{m8} = +40$ mV) cyt *b*-559 measured previously.[1] There is some variability in the pH dependence for hydroquinone reduction of cyt *b*-559 as shown in Fig. 4. We believe that this is caused by the same factors which contribute to variability in the pH dependence of non-cyclic electron transport (see Discussion).

We wish to determine whether it is a low potential or a high potential state of cyt *b*-559 which is oxidized by PS I. Figure 5 shows the pH dependence of the oxidation by 713 nm light of cyt *f* and cyt *b*-559 in spinach chloroplasts in the presence of ascorbate and FMN



Figures 3a,b: Difference spectra for dark oxidation and subsequent reduction of cyt *f* and cyt *b*-559 in spinach chloroplasts, control (a) and with 50 μM antimycin A incubated for 5 min. (b). (a) and (b) Spinach chloroplasts prepared as described above and stored in liquid nitrogen were suspended at 100 μg/ml chlorophyll in 15 mM tricine-KOH, 20 mM NaCl, 4 mM $MgCl_2$, 5 mM phosphate, pH 7·8. (○), oxidation by addition of 60 μM ferricyanide; (●) reduction by addition of 5 mM ferrocyanide; (▲) further reduction by addition of 1 mM ascorbate. Reference wavelength, 540 nm.

as electron acceptor. The amplitude of the cyt *b*-559 response increases by a factor of four from pH 7·0 to 9·0. The smaller increase of a factor 1·5 in the cyt *f* oxidation measured at 554 nm can be attributed to the background of *b*-559 at this wavelength since the half band-width of the *b*-559 is about 8 nm.[1] This monotonic increase with pH in the

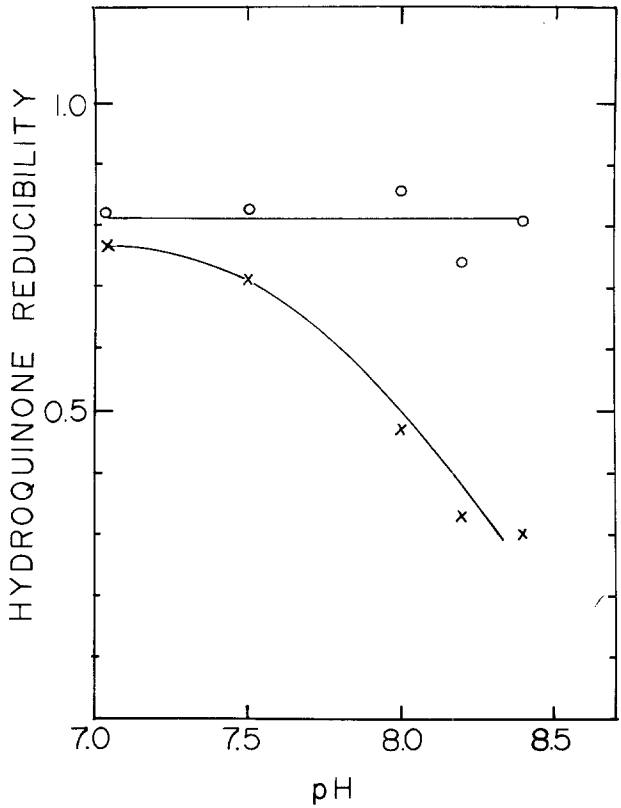


Figure 4. pH dependence of the dark hydroquinone reducibility of cyt *f* and cyt *b*-559 in spinach chloroplasts. At each pH value after 60 μM ferricyanide was added to oxidize the cytochromes in the dark the cytochromes were subsequently reduced by successive additions of 0·1 mM hydroquinone and 1 mM ascorbate. The ordinate shows the fractional reduction by hydroquinone. The reaction medium always contained 10 mM NaCl, 5 mM $MgCl_2$, 5 mM phosphate, and at pH 7·0 and 7·5, 20 mM MOPS buffer; at pH 8·0, 8·2, and 8·4, 20 mM tricine-KOH. The absorbance changes are measured at 551 nm for cyt *f* (○) and 562 nm for cyt *b*-559 (×) in order to minimize the spectral overlap. The ordinate value of 1·0 represents an absorbance change of about 0·004 for 562/540, and 0·0025 for 551/540. Chlorophyll concentration, 90 μg/ml.

cyt *b*-559 oxidation differs from the function described by Ben Hayyim and Avron[9] which had a peak at about pH 7·8. Possibly this is because the *b*-559 becomes more oxidized in the dark at high pH values and the measurements of ref. 9 were made in the absence of ascorbate. A light–dark difference spectrum for the absorbance change measured at pH 8·6 under the conditions of Fig. 5 is characteristic for the photooxidation of cyt *f* and *b*-559 (data not shown).

Comparing Figs. 4 and 5, as the pH is increased the amount of low potential *b*-559 increases, as does the amplitude of the 713 nm light-induced *b*-559 oxidation. This suggests that PS I light oxidizes the low potential cyt *b*-559. This hypothesis is further supported by (a) the slow hydroquinone reduction of cyt *b*-559 photooxidized in the presence of FCCP; and (b) inhibition of cyt *b*-559 photooxidation by the plastoquinone inhibitor DBMIB:

(a) The substituted CCP compounds are known to cause a much enhanced photooxidation of cyt *b*-559 by PS I.[7–9,23] Cramer and Butler have shown that the cyt *b*-559 oxidized by PS I in the presence of *m*-Cl-CCP is reduced by PS II and that the CCP

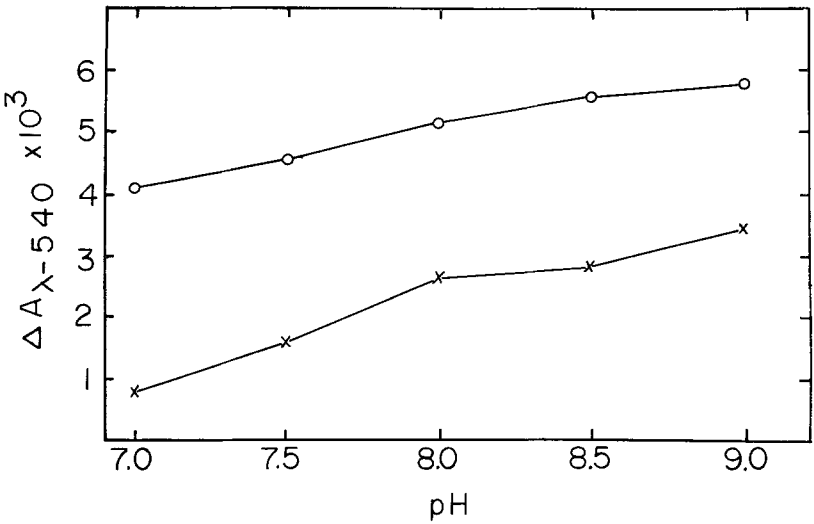


Figure 5. pH dependence of the light-induced oxidation of cyt *f* (o) and cyt *b*-559 (×). Reaction medium contains 25 mM buffer, 5 mM $MgCl_2$, 5 mM phosphate, 3 mM ascorbate, and 25 μM FMN. Buffers: pH 7·0, MOPS; pH 7·5, TES; pH 8·0, tricine-NaOH; pH 8·5 and 9·0, glycylglycine. Chlorophyll concentration, 100 μg/ml. Cyt *f* measured at 554 nm and cyt *b*-559 at 562 nm with 540 nm as reference.

effect is reversed by cysteine.[7] Figure 6a shows the stimulation of cyt *b*-559 photooxidation by 713 nm light in spinach chloroplasts at pH 8·0 by FCCP (3 μM). After the actinic light is turned off the rate of dark reduction in the presence of hydroquinone is slow. Ascorbate causes a further reduction. Figures 6b, c, show that it is only the photooxidized cyt *b*-559 which is reduced slowly by hydroquinone. Hydroquinone reduction of the *b*-559 oxidized by ferricyanide at pH 8·0 in the presence of FCCP is much faster (Fig. 6b), as is the hydroquinone reduction of photooxidized cyt *f* measured at 554 nm (Fig. 6c). The small amplitude of cyt *f* photooxidation in the absence of an exogenous electron acceptor and FCCP is repeatedly found in these chloroplast preparations, and may be due to a smaller pool of endogenous acceptor. It should be noted that the rate of hydroquinone reduction of chemically oxidized cytochrome is pH dependent, and is more rapid at pH 8·0 (Fig. 6b) than at pH 7·0 (Fig. 1), presumably because of a pH dependence of hydroquinone accessibility. The comparison of Fig. 6a with Figs. 6b and c, with all three experiments performed at the same pH, leads to the conclusion that it is low potential *b*-559 which is photooxidized by PS I in the presence of FCCP, and that the slow

hydroquinone reduction of the photooxidized cyt *b*-559 in Fig. 6a is a measure of the dark conversion of low potential *b*-559 back to high potential *b*-559. It is of course possible that light and FCCP might cause a structural change in the membrane whose effect is to make cyt *b*-559 and not cyt *f* inaccessible to hydroquinone. But Fig. 2 shows that FCCP at high concentrations in the dark can irreversibly convert high potential *b*-559

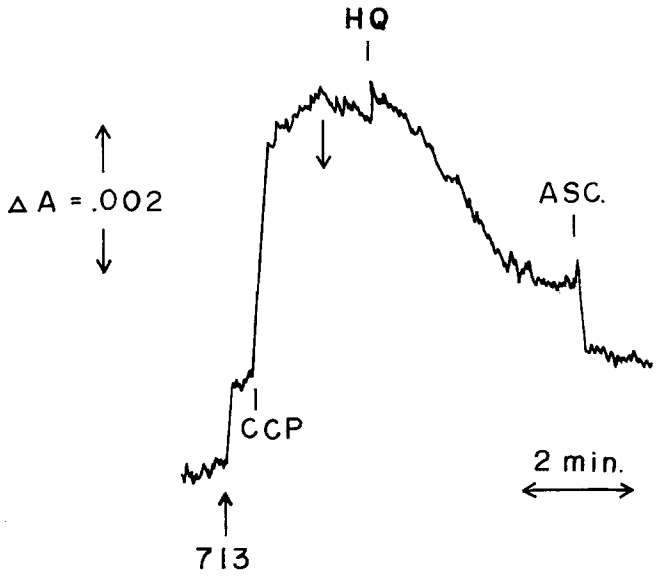


Fig. 6a

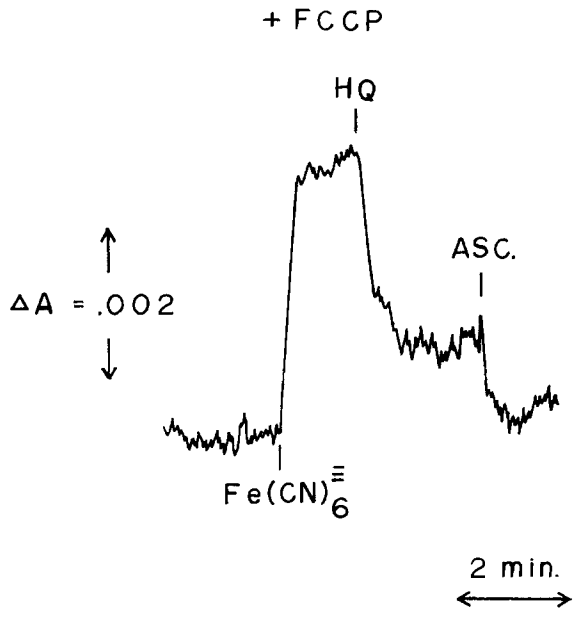


Fig. 6b

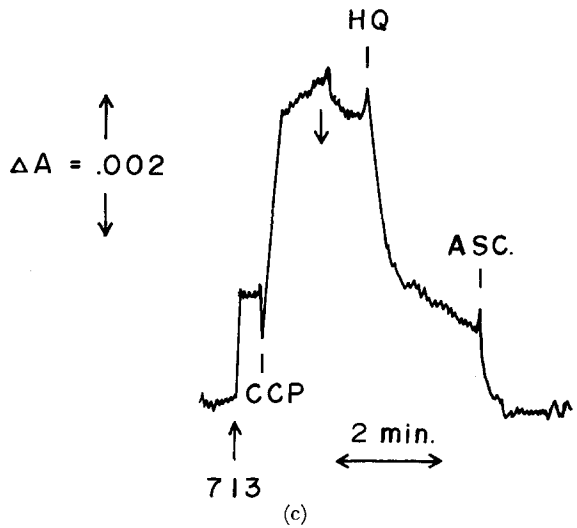


Figure 6. (a) Hydroquinone reduction of cyt *b*-559 oxidized by far-red light in the presence of FCCP; (b) hydroquinone reduction of cyt *b*-559 oxidized by ferricyanide in the presence of FCCP; (c) hydroquinone reduction of cyt *f* oxidized by 713 nm light in the presence of FCCP. Reaction medium: 25 mM tricine-NaOH, pH 8·0, 5 mM $MgCl_2$, 5 mM P_i. Chlorophyll concentration, 100 μg/ml; hydroquinone, 0·1 mM; sodium ascorbate, 2 mM; ferricyanide, 0·1 mM; FCCP, 3 μM. Cyt *f* measured at 554 nm and cyt *b*-559 at 562 nm with 540 nm reference. Upward deflection represents a decrease in absorbance.

to low potential *b*-559, and it is assumed that in the presence of light this can occur reversibly with lower concentrations of FCCP.

Since the concentration of low potential *b* present in the dark (Fig. 6b) is much less than the amount of *b*-559 photooxidized in Fig. 6a in the presence of FCCP, it is concluded that FCCP facilitates the photoconversion of high potential to low potential *b*-559 and that this is the explanation of the stimulatory effect of CCP compounds on *b*-559 photooxidation.

The concentration of FCCP used in the experiments of Fig. 6, 3 μM, is significantly larger than the minimum amount needed to stimulate the *b*-559 photooxidation, which is on the order of 10^{-7} M (see Fig. 8). In order to test the effects of hydroquinone and ascorbate as reductants for *b*-559 it is necessary to inhibit the endogenous dark reduction of oxidized *b*-559. 3 μM FCCP is the minimum concentration which would sufficiently inhibit this endogenous reduction. Antimycin A also stimulates the photooxidation of *b*-559 by PS I,[8] and we propose that this can also be ascribed to enhanced photoconversion of high potential to low potential *b*-559, as suggested by Fig. 3. However, we have not been able to test this hypothesis, as antimycin A does not inhibit the dark endogenous reduction of photooxidized cyt *b*-559 (data not shown).

(b) The inhibition of non-cyclic and cyclic electron transport and phosphorylation by the quinone analog DBMIB can be reversed by plastoquinone.[24,25] It has been shown that DBMIB blocks the reduction of cyt *f* by PS II light and the photooxidation of cyt *b*-559 by PS I. Both of these inhibitory effects could be reversed by plastoquinone addition, implying that plastoquinone mediates electron transport between cyt *b*-559

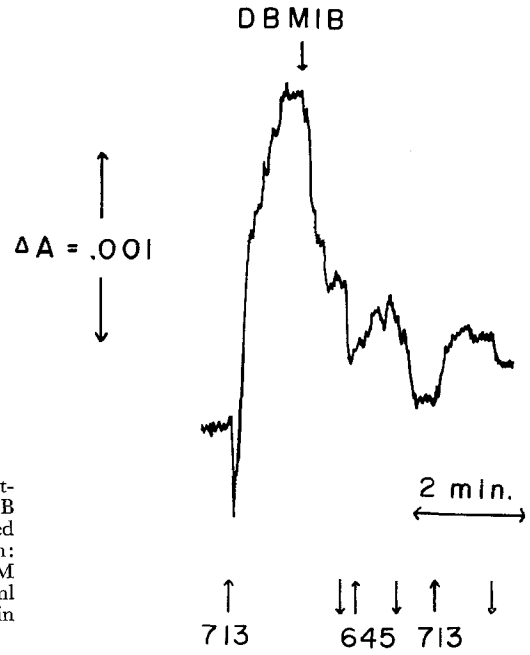


Figure 7. Effect of DBMIB (2 μM) on the light-induced absorbance changes of cyt *b*-559. DBMIB added during 713 nm illumination. Cyt *b*-559 measured at 562 nm with 570 reference. Reaction medium: 25 mM tricine-NaOH, pH 8·0, 5 mM $MgCl_2$, 5 mM Na_2HPO_4, 25 μM FMN, chloroplasts at 100 μg/ml chlorophyll. Upward deflection represents a decrease in absorbance.

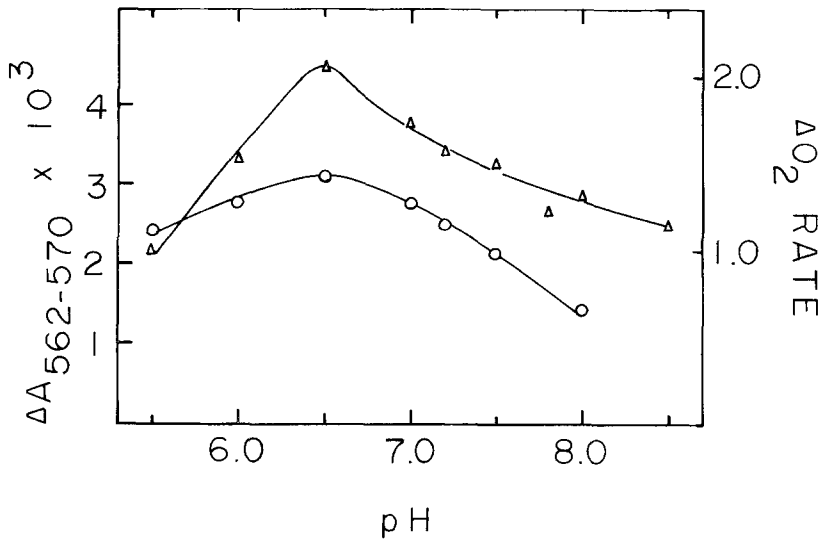


Figure 8. Comparison of the pH dependence of the light-induced cyt *b*-559 oxidative absorbance change with FCCP (○) and the increase in rate of O_2 evolution caused by FCCP (△). FCCP stimulated O_2 evolution expressed as ratio ± FCCP. Reaction medium contains 25 mM buffer, 5 mM $MgCl_2$ and 5 mM Na_2HPO_4. Buffers: pH = 5·5, 6·0, 6·5, MES; 7·0, 7·2, MOPS; 7·5, TES; 8·0, 8·5, tricine-NaOH. FCCP and chlorophyll concentrations, 0·1 μM and 100 μg/ml, respectively, for absorbance measurements, 0·3 μM and 18 μg/ml for O_2 measurements. Cyt *b*-559 oxidation induced by 713 nm actinic light with no added acceptor, no ascorbate.

and cyt *f*.[17] The cyt *b*-559 experiments discussed in reference 17 were done in the presence of FCCP. Figure 7 explicitly shows that DBMIB also inhibits the photooxidation of cyt *b*-559 in the absence of FCCP, so that DBMIB inhibition of the *b*-559 photooxidation is not dependent upon FCCP. If, on the basis of this experiment and those of ref. 17, it is assumed that plastoquinone is required for the photooxidation of cyt *b*-559, and that the E_{m7} of plastoquinone A is about +110 mV,[26] then the thermodynamics very definitely indicates that it must be a low potential form ($E_{m7} \leqslant 100$ mV) of cyt *b*-559 which is photooxidized by PS I.

Discussion

There is general agreement that cyt *b*-559 is structurally close to PS II since it is found in the detergent separated PS II particle fraction,[27,28] it is oxidized by PS II at 77° K,[10–13] and it is readily reduced by PS II at room temperature.[4,6,7,9] The pathway of the cyt *b*-559 photooxidation at room temperature is the most poorly understood. This is because the only conditions under which one observes a large photooxidation (e.g., low temperature, Tris-treatment without donor addition, or FCCP addition) preclude electron transport coupled to phosphorylation. At 77° K, where thermochemical reactions including water splitting and electron transfer from PS II to PS I would be inhibited, PS II preferentially oxidizes the *b*-559.[10–13] PS II also preferentially oxidizes the *b*-559 when the water splitting reaction is inactivated by Tris-treatment.[10] The preferential PS II oxidation of what appears to be high potential *b*-559[13] has been observed only under conditions where water cannot serve as electron donor to PS II[11] and, in the case of the measurements at 77° K, where electron flow from PS II to PS I is inhibited. Thus, there is some question as to whether this pathway coupled to the PS II reaction center functions under physiological conditions.

Figures 4–7 above lead to the conclusion that the cyt *b*-559 oxidized by PS I is low potential ($E_m \leqslant 100$ mV). The problem here is that the amplitude and rate of the photooxidation are appreciably smaller than for cyt *f* under conditions of basal or phosphorylating electron transport.[7,8] Thus, in the absence of FCCP or compounds with similar effect on *b*-559, it is not known whether the *b*-559 really turns over to a significant extent on the pathway from PS II to PS I. We can say from the experiments presented here that if any cyt *b*-559 is oxidized by PS I, then it is low potential *b*-559. The question remains as to whether any *b*-559 is oxidized by PS I under well-coupled conditions.

If low potential cyt *b*-559 does function to a significant extent in the pathway from PS II to PS I, then the following must also occur: (a) high potential *b*-559 must be converted to low potential *b*-559 in the light since apparently there is not enough of the latter present in the dark at pH 7–8 to mediate electron flow between PS II and I (Figs. 1 and 4); (b) the rate constant for PS II reduction of the cyt *b*-559 must be much greater than that for oxidation, since far-red light has such a small net oxidative effect on the *b*-559. The latter condition is reasonable since the *b*-559 appears to be closely linked to PS II (see Fig. 9), and the most likely discrete phosphorylation site, a site of relatively slow electron transfer, is on the PS I side of low potential *b*-559. It will not be possible to say that low potential *b*-559 is in the chain under coupled conditions unless these auxiliary points can be experimentally documented. We feel that it is likely that cyt *b*-559 functions in the electron transport chain connecting PS II and PS I because of the following reasons: (a) The analogy with mitochondrial electron transport men-

tioned above in the introduction; (b) other pathways suggested for the cyt *b*-559[4] seem more complicated and less likely; (c) the possible regulatory role of cyt *b*-559 as an electron transport component which can assume two different redox states, a low potential (+80 mV) state which allows it to donate to plastoquinone, and a high potential (+350 mV) state from which electrons thermodynamically cannot be transferred to plastoquinone.

The qualitative correlation between the increase in amplitude of *b*-559 photooxidation (Fig. 5) and the well-known increase in rate of non-cyclic electron transport up to approximately pH 8·5[29] suggests that the redox state of cyt *b*-559 could be involved

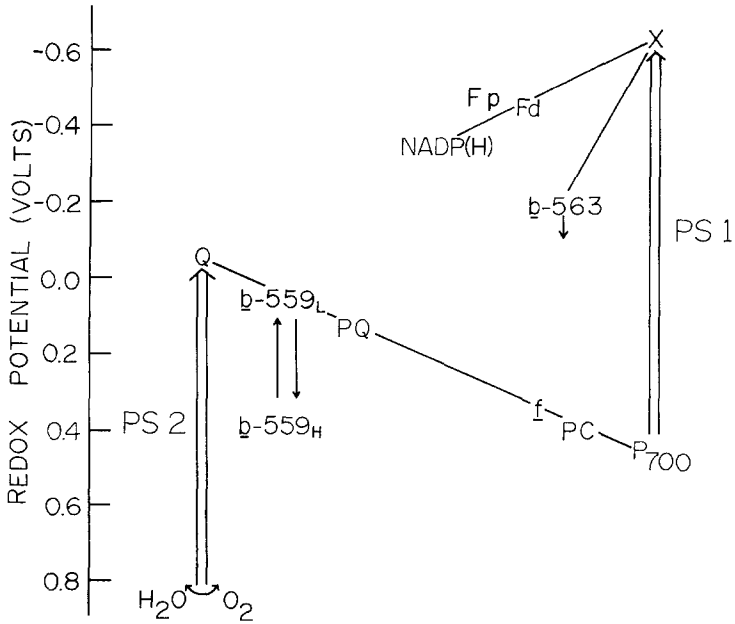


Figure 9. The Z-scheme of photosynthetic electron transport. b-559$_L$ and b-559$_H$ represent, respectively, the low and high potential forms of the cyt *b*-559. The position of plastiquinone (PQ) in the scheme is based on the experiments with DBMIB (Fig. 7 above and ref. 17).

in regulation of non-cyclic electron transport. In an experiment done in parallel with that of Fig. 5, we find that the Hill reaction activity with ferricyanide and ADP has a broad peak centered around pH 8·5 (data not shown). The decrease in O_2 evolution at pH values above the optimum cannot be attributed to cyt *b*-559, but must involve other PS II reactions. An approximate pH correlation between the overall rate of non-cyclic electron transport and the amplitude of the PS I mediated photooxidation of cyt *b*-559 can also be seen in the presence of FCCP. The stimulation of the O_2 evolution rate by FCCP has a peak at pH 6·5. The amplitude of the *b*-559 photooxidation by PS I also has a broad peak around pH 6·5 (Fig. 8). We note that we were only able to obtain this correlation by using slightly different concentrations of FCCP in the two experiments.

Figures 5 and 8 suggest that the rate of non-cyclic electron transport could be partly regulated by the thermodynamic state of cyt *b*-559. When there is an excess of the high

potential form of cyt *b*-559, the rate of non-cyclic electron flow will be low as it is at low pH. As more low potential cyt *b*-559 is formed, the rate will accelerate. An excess of low potential *b*-559 appears to be synonymous with an uncoupled state (Figs. 2, 3) so that the optimum state for phosphorylation may represent a mixture of approximately equal amounts of low and high potential *b*-559, which from Fig. 4 would occur around pH 8·0. The position of cyt *b*-559 in the Z-scheme model of photosynthetic electron transport is shown in Fig. 9. Two states of a cyt *b*-559, low potential (L), and high potential (H), are shown in the model with only the low potential state being able to reduce plastoquinone, the next carrier in the chain. We have formulated this model in terms of only two redox states for cyt *b*-559 as the simplest interpretation of our data. The data itself does not exclude more redox states with potentials between those of ascorbate and hydroquinone.

There are two different redox states of a cyt *b* component in rat liver and pigeon heart mitochondria. The presence of the high potential component ($E_{m7} = +245$ mV) is dependent upon addition of ATP (45 mM). In the absence of ATP, or in uncoupled mitochondria, the E_{m7} of the low potential component is about −35 mV.[15,16] Thus, the redox potential difference between the high and low potential *b* cyt components is about 300 mV in both mitochondria and chloroplasts. One difference, however, is that the high potential *b*-559 in chloroplasts is present in the dark without addition of any energy source. Thus, if the high potential *b*-559 is an energized form of the cyt *b*-559, it is not the high energy state which can be directly utilized for phosphorylation, as has been proposed for the high potential cyt b_T of mitochondria.[16] The thermodynamic mechanism for control of non-cyclic electron transport by cyt *b*-559 proposed here is similar to that proposed previously for regulation of respiratory electron transport.[16] However, the inhibited state for chloroplasts discussed here, in which forward electron transport tends not to occur from cyt $b\text{-}559_H$ ($E_{m7} = +350$ mV) to plastoquinone ($E_m \approx +110$ mV) seems better defined thermodynamically than the analogous state for mitochondria with cyt b_T at +250 mV and cyt c_1 at +220 mV.

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