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HIGH-POTENTIAL CYTOCHROME *b*-559 AS A SECONDARY QUENCHER OF CHLOROPLAST FLUORESCENCE IN THE PRESENCE OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA

W. A. CRAMER AND H. BÖHME

Department of Biological Sciences, Purdue University, Lafayette, Ind. 47907 (U.S.A.)

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SUMMARY

Carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), antimycin A, and hydroxylamine cause an increase in the fluorescence yield of chloroplasts observed with a weak exciting light in the presence of DCMU. Under these conditions the three compounds cause an increase in the time of decay of the fluorescence yield change induced by actinic light, and as well convert hydroquinone-reducible high-potential *b*-559 ($E_{m7} \approx +350$ mV) to a low potential form which is reducible by ascorbate, but not by hydroquinone. The concentration dependence of the two fluorescence parameters and the cytochrome *b*-559 potential change is the same for antimycin A and is almost the same for FCCP.

The most likely explanation of these effects seems to be that high-potential cytochrome *b*-559, but not low potential *b*-559, can accept electrons from the primary acceptor of system II in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Together with data obtained in other laboratories on the low-temperature photooxidation of high potential *b*-559, this suggests the possibility of a cyclic pathway operating around Photosystem II. This pathway is probably not physiologically significant in the light reactions of photosynthesis, as the fluorescence experiments indicate it saturates at low light intensities.

Electron transport from water to methyl viologen is not inhibited by 2 μM FCCP or 0.6 mM hydroxylamine over times in which most of the high-potential *b*-559 is converted to a low-potential form, showing that high-potential *b*-559 cannot be in the pathway of water oxidation.

INTRODUCTION

Redox titration data indicate that there are two different redox states of the chloroplast cytochrome *b*-559, a high-potential form with $E_{m7} \approx +350$ mV[1-4] and a low-potential form with $E_{m7} \approx +80$ mV[4-6]. It has been shown that when cytochrome *b*-559 is oxidized by Photosystem I it is the low-potential form which is oxidized[7,8]. It is not proven that such oxidation occurs under physiological conditions. The high-potential cytochrome *b*-559 is oxidized by System II light at 77°

Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; E_{m7}, midpoint potential at pH 7; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; S-13, 5-chloro-3-*tert.*-butyl-2'-chloro-4'-nitrosalicylanilide; TTFB; tetrachloro-2-trifluoromethyl benzimidazole.

K[3,9–12], from which it has been inferred that cytochrome *b*-559 operates in a 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-sensitive pathway between two System II photoreactions[2], or cytochrome *b*-559 accepts the first of the four electrons transferred from water in the oxygen evolution pathway[11].

Evidence presented here indicates that high-potential *b*-559 is in a pathway which can oxidize the primary acceptor of Photosystem II in the presence of DCMU. This pathway saturates at low light intensities.

METHODS

(1) *Chloroplast preparation*

Spinach was grown in a greenhouse or in a controlled climate facility. Chloroplasts were made by homogenizing 15 g of leaves for 2 sec in a Sorvall Omnimixer in 50 ml of pH 7.0 medium containing 0.4 M sorbitol, 0.05 M morpholinopropane sulfonate–NaOH, 0.01 M NaCl, 5 mM MgCl, 2 mM EDTA, 1 mM $MnCl_2$, and 2 mM sodium ascorbate added just before use. The homogenate was filtered through eight layers of cheesecloth and one layer of 40 μm mesh nylon and then centrifuged for 1 min at 4000 $\times$ *g*. The chloroplast pellet was resuspended in 1 ml of medium at pH 7.8 containing 0.4 M sorbitol, 0.05 M Tricine–NaOH, 0.01 M NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 1 mM $MnCl_2$, and 1 % crystalline bovine serum albumin added just before use.

(2) *Chloroplast coupling*

O_2 uptake was measured with a YSI 5331 electrode in a reaction medium at pH 7.8 containing 25 mM Tricine–NaOH, 5 mM $MgCl_2$, 5 mM K_2HPO_4, together with 0.1 mM methyl viologen and 0.2 mM NaN_3. The intensity of heat-filtered white light was $3 \cdot 10^5$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}. Stimulation of O_2 uptake was measured with two successive additions of a small (60 μM) amount of ADP, with the first and second additions increasing the rate by a factor of about 1.5 and 3.0, respectively. This degree of coupling was stable for at least 3 h.

(3) *Fluorescence yield measurements*

Fluorescence was excited with chopped monochromatic 652 nm light of intensity 75 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}, defined by a Bausch and Lomb high intensity monochromator and an interference filter. The wavelength of the actinic light was 650 nm. The apparatus was otherwise as described previously[13]. The fluorescence and cytochrome absorbance measurements were made in a medium consisting of 25 mM Tricine–KOH, 5 mM $MgCl_2$, 5 mM K_2HPO_4, pH 7.8, under aerobic conditions.

(4) *Measurement of cytochrome absorbance changes*

The technique and apparatus have been described before[5,7,8] except that measurements at a lower chlorophyll concentration and higher amplification were made possible by the use of a higher chopping speed (480 cycles/sec) and magnetic stirring. All cytochrome absorbance measurements were made with 25 μg/ml chlorophyll in a 3-ml 1-cm^2 cuvette where the light intensity was 1–1.5 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}. The reference wavelength was 540 nm, with a half-bandwidth of 1.4 nm. The wavelength of the actinic light was 643 nm with an intensity of 85 ergs$\cdot$cm$^{-2}\cdot$sec^{-1} in order to match the conditions of the fluorescence yield experiments.

(5) *Uncouplers*

We are grateful to Dr. P. G. Heytler for the carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), to Dr. P. C. Hamm for the 5-chloro-3-*tert.*-butyl-2'-chloro-4'-nitrosalicylanilide (S-13) and to Dr. R. B. Beechey for the tetrachloro-2-trifluoro-

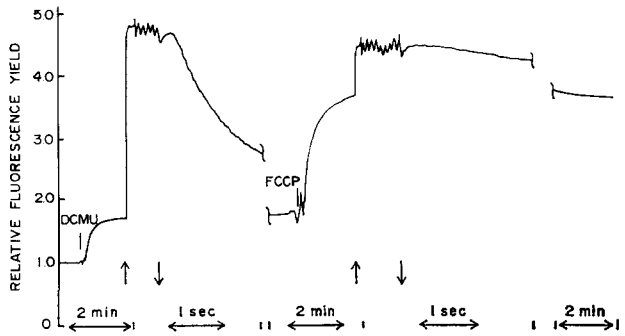


Fig. 1. Effect of FCCP in the presence of DCMU on the fluorescence yield in the measuring light and on the decay rate of the actinic light-induced fluorescence yield increase. DCMU, 1 μM; FCCP, 0.5 μM. Upward arrows, actinic light on; downward arrows, actinic light off. Actinic light intensity, $3 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}. Amplifier time constant during actinic illumination, 1 sec; time constant in measuring light, 0.1 sec.

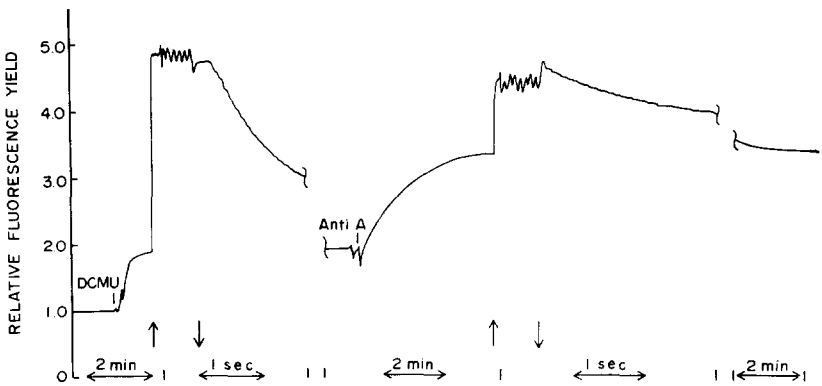


Fig. 2. Effect of antimycin A (39 μM) on the measuring light fluorescence yield and decay rate after illumination in the presence of DCMU. Conditions as in Fig. 1.

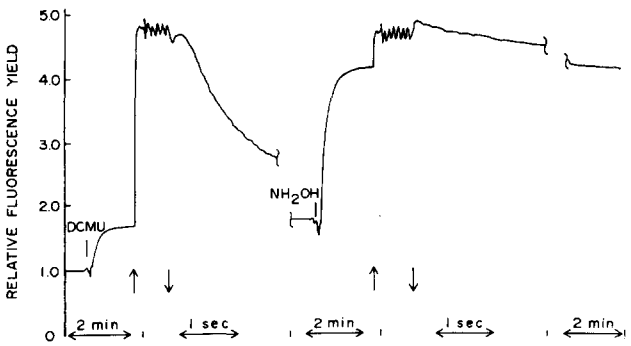


Fig. 3. Effect of hydroxylamine (NH_2OH) on the measuring light fluorescence yield and decay rate after illumination in the presence of DCMU. Conditions as in Fig. 1; hydroxylamine, 0.2 mM.

methyl benzimidazole (TTFB). Antimycin A was obtained from Calbiochem., and its concentration was measured using a millimolar extinction coefficient of 4.8 at 320 nm[14].

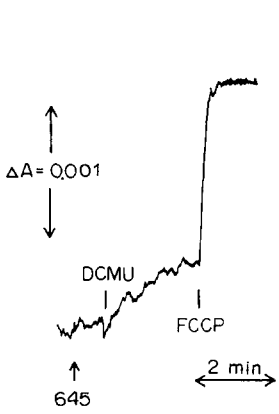
RESULTS AND DISCUSSION

The increase of fluororescence yield caused by DCMU (1 μM) addition in the measuring light of intensity 75 ergs·cm^{-2}·sec^{-1} is only 20–25 % of the maximum increase in fluoresence yield caused by actinic light of intensity $3 \cdot 10^4$ ergs·cm^{-2}·sec^{-1} (Figs. 1–3). This DCMU concentration inhibits electron flow to methyl viologen by 95 % with the same reaction medium, chlorophyll concentration, and actinic light intensity used in the fluorescence yield experiments. The effect of DCMU on the fluorescence yield in the measuring light is significantly increased by addition of FCCP (Fig. 1), antimycin A (Fig. 2), and hydroxylamine (Fig. 3). The three compounds were added in Figs. 1–3 at concentrations of 0.5 μM, 39 μM, and 0.2 mM, respectively. The fluorescence yield increase caused by adding any of the three compounds at these concentrations in the absence of DCMU was negligible. As shown in Figs. 1–3, all three compounds cause an increase in the time it takes for the fluorescence yield to decay back to the level in the measuring light after the actinic light is turned off.

A very similar effect of FCCP and 2,4-dinitrophenol on measuring light fluorescence yield in whole cell of *Porphyridium cruentum* and *Chlamydomonas reinhardi* in the presence of DCMU has been reported by HOCH AND RANDLES[15]. They attributed the effects of FCCP and 2,4-dinitrophenol to their property of increasing membrane permeability to protons. BENNOUN[16] has shown that the decay time in the dark of the light-induced fluorescence yield increase with DCMU is increased by hydroxylamine. From the evidence presented below it is our contention that these effects on fluorescence of FCCP and hydroxylamine, as well as antimycin A, can be explained by all of these agents causing a conversion of high-potential *b*-559 to a low-potential form, with only the former being able to act as a secondary quencher of fluorescence in the presence of DCMU. As high-potential *b*-559 is converted to a lower-potential form with DCMU present, this hypothesis would predict that (a) the fluorescence yield in the measuring light will increase since the only pathway for "Q" oxidation in the presence of DCMU is being depleted, and (b) the time it takes for the fluorescence to decay from a high actinic light-induced value back to the value in the measuring light should increase in a parallel fashion.

We have previously found that FCCP and antimycin A at relatively high concentrations in the dark can irreversibly convert high-potential *b*-559 to low-potential *b*-559[8]. At lower concentrations of FCCP high-potential *b*-559 is reversibly converted to a low-potential form in the light[8]. For FCCP we consider that the amplitude of the Photosystem I-mediated photooxidation is a measure of the amount of conversion of high-potential to low-potential *b*-559. The effect of FCCP in converting high-potential *b*-559 to a low-potential form under the conditions of the fluorescence experiment in Fig. 1 was determined by measuring the photooxidation of *b*-559 under conditions as similar as possible to those of Fig. 1. That is, the chlorophyll concentration in both cases was 25 μg/ml and 645 nm light with an intensity of 85 ergs·cm^{-2}·sec^{-1} was used as the actinic source for cytochrome absorbance changes. DCMU and FCCP were successively added in the cytochrome experiment

at time intervals approximating those in Fig. 1 (Fig. 4). In the presence of DCMU most of the cytochrome *f* will be oxidized by the 645-nm light acting on System I. For this reason the difference spectrum for the FCCP-induced absorbance decrease has a well-defined maximum at 559 nm (Fig. 5) and the amplitude of this absorbance decrease at 561 nm relative to 540 nm as shown in Fig. 4 is used as a measure of the *b*-559 photooxidation. The *b*-559 change is measured at 561 nm just to minimize any residual contribution of cytochrome *f*. Cytochrome *b*-563 is oxidized in the dark at the start of the experiment[17] and will not contribute to the photooxidation.



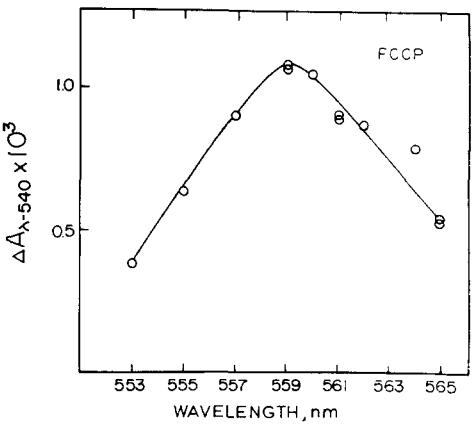


Fig. 4. Oxidative absorbance change of cytochrome *b*-559, measured at 561 nm, upon addition of 1 μM FCCP in the presence of 1 μM DCMU and 85 ergs·cm^{-2}·sec^{-1} of red light. Conditions otherwise as in METHODS (*4*).

Fig. 5. Light–dark difference spectrum for cytochrome *b*-559 oxidation in the presence of FCCP (1 μM) and DCMU (1 μM). Conditions otherwise as in METHODS (*4*).

If anything photoreduction of *b*-563 under these conditions might cause a decrease in the amplitude of the *b*-559 photooxidation measured at 561 nm and for this reason it is thought that the point above the curve at 564 nm in Fig. 5 is just a consequence of experimental error. 645 nm light was used in Fig. 4 and in similar experiments to obtain the data of Figs. 6 and 7 just in order to reproduce the conditions of the fluorescence experiments. In the presence of DCMU it is assumed that 645 nm light acts only on Photosystem I, since photooxidation of cytochrome *b*-559 by System II light has thus far only been observed at low temperature[9–12] in Tris-washed chloroplasts[9], or at very high pH[2], that is when the dark reactions of System II have been impeded or inactivated. In the presence of FCCP it has been clearly shown that cytochrome *b*-559 is oxidized by System I light and reduced by System II light[7,17].

The amplitude of the *b*-559 photooxidation was measured as a function of FCCP concentration in the presence of 1 μM DCMU using conditions as closely identical as possible to those of the fluorescence yield experiments. Fig. 6 shows as a function of the concentration of added FCCP (1) the amplitude of the actinic light-induced *b*-559 oxidation, (2) the increase of the measuring light fluorescence with DCMU, and (3) the increase in decay time of the fluorescence yield. The concentration dependence of the *b*-559 photooxidation and the decay time increase

are quantitatively similar. The effect of FCCP on the measuring light fluorescence is also similar, but this function is offset somewhat from the other two.

It has been shown that antimycin A in the dark can convert hydroquinone reducible high-potential *b*-559 to a low-potential form which can be reduced by ascorbate, but not by hydroquinone[8]. Fig. 7 shows the decrease in hydroquinone

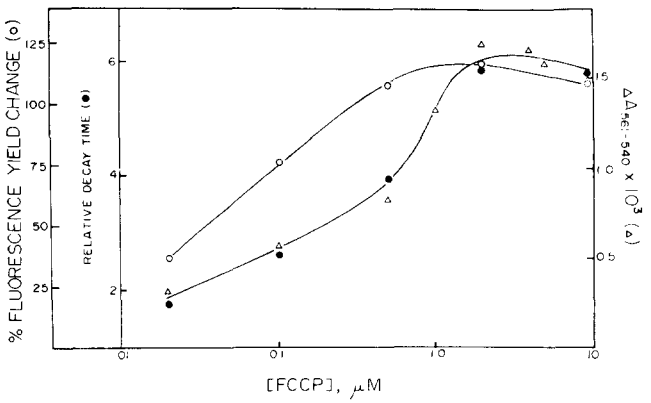


Fig. 6. The change as a function of FCCP concentration of (a) measuring light fluorescence yield (○); (b) decay time of actinic light-induced fluorescence yield increase (●); and (c) 85 ergs·cm^{-2}·sec^{-1} light-induced oxidation of cytochrome *b*-559 (△). (a) and (b) as in Fig. 1 with 1 μM DCMU; (c) as in Fig. 4. FCCP concentrations plotted on a logarithmic scale.

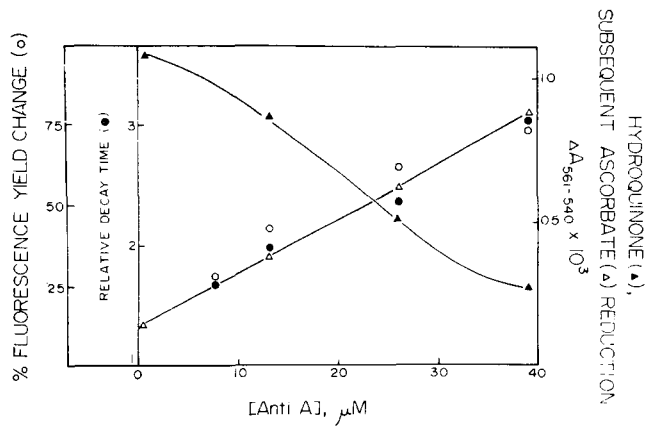


Fig. 7. The change as a function of antimycin A concentration of (a) measuring light fluorescence (○); (b) decay time (●); and (c) hydroquinone reducible and non-reducible cytochrome *b*-559. (a) and (b) as in Fig. 2; (c) cytochrome *b*-559 measured at 561 nm was oxidized with 100 μM ferricyanide and the reduction was determined with a subsequent addition of 0.2 mM hydroquinone (▲), and then with a further addition of 1 mM ascorbate (△), as shown in ref. 8.

reducibility and increase in reduction by ascorbate subsequently added, as a function of antimycin A concentration. The function describing the increase in ascorbate reduction is very similar to those describing the increase in measuring light fluorescence and the increase in decay time.

ERIXON AND BUTLER[12] have found that high concentrations (25 mM) of hydroxylamine cause an oxidation of the cytochrome *b*-559[12]. Lower concentrations

of hydroxylamine convert high-potential *b*-559 to a low-potential form in the dark. The comparison of the amount of low-potential *b*-559, the increase in measuring light fluorescence, and the increase in decay time (Fig. 8) shows that all three functions generally increase with hydroxylamine concentration, but that the shape of the functions are dissimilar. The slope of the function describing the increase in measuring

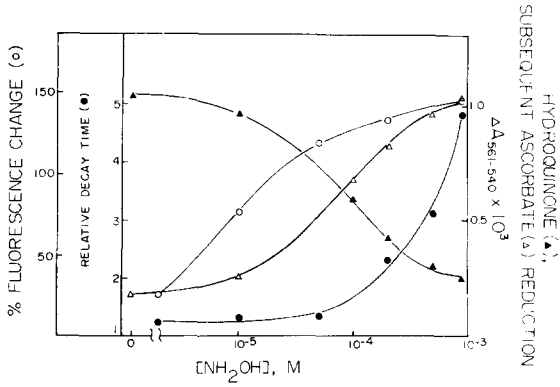


Fig. 8. The change as a function of hydroxylamine concentration of (a) measuring light fluorescence (○); (b) decay time (●); and (c) cytochrome *b*-559 measured a 561 nm successively reduced by addition of 0.2 mM hydroquinone (▲) and 1 mM ascorbate (△), after oxidation by 100 μM ferricyanide, as in Fig. 6. Hydroxylamine concentrations plotted on a logarithmic scale.

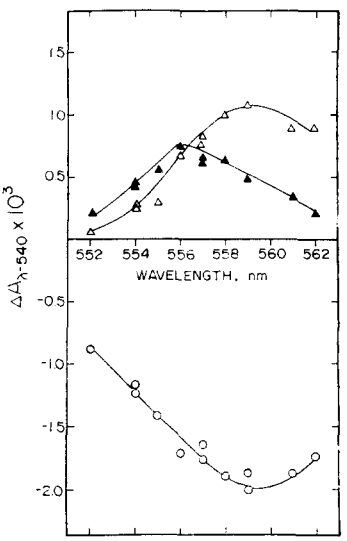


Fig. 9. Difference spectra for the dark oxidative and reductive absorbance changes in the presence of hydroxylamine (0.6 mM). Oxidation by 150 μM ferricyanide (○). Successive reduction by 0.25 mM hydroquinone (▲) and 1.5 mM ascorbate (△).

light fluorescence is greatest at low hydroxylamine concentration, though at these concentrations the decay time increases only slightly from the control value. Between 0.1 and 1.0 mM hydroxylamine, the decay time increases greatly. The slower rise in the cytochrome *b*-559 function in Fig. 8 may be due to the method of assay which involves first oxidizing the cytochrome with ferricyanide in the presence of hydroxylamine in order to determine the extent of the hydroquinone and ascorbate reduction. The ferricyanide oxidizes the hydroxylamine, decreasing its effective concentration. This does not explain, however, why the decay time function for hydroxylamine is so different, since it is measured in the same experiment as the fluorescence increase. The explanation of the latter discrepancy may reside in the known ability of hydroxylamine to affect Photosystem II activity[18,19]. In particular, if hydroxylamine can efficiently donate electrons to Photosystem II under these experimental conditions this would explain why the fluorescence increase rises faster than the increase in decay time as a function of hydroxylamine concentration. The extra source of electrons would result in a more reduced "Q". There do not seem to be any inhibitory effects of hydroxylamine on Photosystem II in these experiments (see Fig. 12), perhaps because there is no preincubation[19]. Fig. 9 shows the difference spectra for the dark chemical oxidation by ferricyanide in the presence of hydroxylamine, and the successive reduction by hydroquinone and ascorbate. The peaks of the spectra for ferricyanide oxidation and ascorbate reduction are close to 559 nm. The effect of hydroxyl-

amine in lowering the potential of the *b*-559 is shown in the peak of the spectrum for hydroquinone reduction being at 556 nm, with cytochrome *f* which is always hydroquinone reducible contributing more to the peak than high-potential *b*-559 and in the greater amplitude of ascorbate reduction. In the control with hydroxylamine the spectrum for hydroquinone reduction shows a 10% increase in amplitude from 555 to 559 nm, and the reduction caused by hydroquinone addition at 561 nm is 5 times that caused by subsequent ascorbate addition (data not shown).

In the case of hydroxylamine, it was necessary to measure the change of potential of the *b*-559 by a dark chemical method, since we find hydroxylamine does not stimulate the photooxidation of cytochrome *b*-559 by Photosystem I. In this respect the action of the hydroxylamine on the *b*-559 is different from FCCP. The conversion of *b*-559 to a lower-potential form by FCCP ($\lesssim 5\ \mu M$) requires light, which also causes photooxidation. The properties of antimycin A in this respect are intermediate between those of FCCP and hydroxylamine. Antimycin A between 10 and 40 μM causes a conversion of the *b*-559 to a lower-potential form in the dark, and also stimulates the photooxidation of the *b*-559, though the amplitude of this photooxidation is less than for FCCP. Thus the low-potential forms of the cytochrome *b*-559 induced by FCCP, antimycin A, and hydroxylamine do not necessarily have the same midpoint potentials or ligand environment. If the midpoint potentials are similar, then the compounds must have other effects on the cytochrome or other parts of the electron transport chain. But as far as increasing the fluorescence yield and the fluorescence decay time, FCCP, antimycin A, and in a qualitative sense, hydroxylamine as well, all have the common property of decreasing the concentration of high-potential *b*-559. The presence of high-potential *b*-559 leads to a lower fluorescence yield in the presence of DCMU. Assuming that the fluorescence yield is high in the presence of DCMU because reoxidation of the "Q" by the main electron transport chain is blocked[20], this implies that high-potential *b*-559 can accept electrons from the "Q" in the presence of DCMU. At low light intensities the cytochrome *b*-559 might then operate in a cyclic pathway around Photosystem II as shown in Fig. 10, since photooxidation of high-potential *b*-559

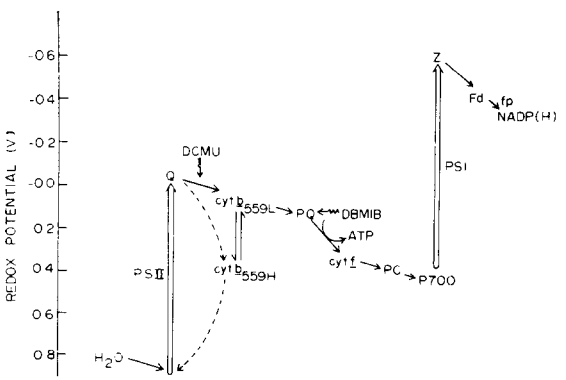


Fig. 10. Scheme for non-cyclic photosynthetic electron transport. The role for high- and low-potential states of cytochrome *b*-559 in electron transport and the placing of cytochrome *b*-559 on the System II side of plastoquinone is discussed in ref. 8. The phosphorylation site between plastoquinone and cytochrome *f* is discussed in ref. 29.
Abbreviations: PSI, Photosystem I; PSII, Photosystem II, PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin; Fp, flavoprotein; DBMIB, 2,5-dibromo-3-methyl-6-isopropylbenzaquinone.

has been demonstrated at liquid-nitrogen temperatures where the dark reduction is blocked[3,9–12]. It is known that at moderate light intensities the fluorescence yield saturates in the presence of DCMU. Fig. 11 shows the fluorescence yield measured as a function of light intensity in the presence and absence of DCMU, and in the absence of an electron acceptor. At saturating actinic light intensity the fluorescence yield is increased by a factor of 4.5 over the value in the measuring light at 75 ergs·cm^{-2}·sec^{-1}. In the absence of DCMU a light intensity of $6 \cdot 10^3$ ergs·cm^{-2}·sec^{-1} is required for half of the maximum fluorescence yield increase. In the presence of DCMU half of the maximum increase is obtained at a light intensity of 250 ergs·cm^{-2}·sec^{-1}.

It has not been possible to obtain direct proof of electron flow through high-potential cytochrome *b*-559 in the presence of DCMU. The experimental problems seem to be interference due to photooxidation of low-potential *b*-559 by Photosystem I and preferential photoreduction by "Q", rather than high-potential *b*-559, of ferricyanide added to oxidize the high-potential *b*-559. Although the inability to detect Photosystem II reduction of cytochrome *b*-559 in the presence of DCMU can be explained, it is partly for this reason that other hypotheses for the fluorescence changes caused by FCCP, antimycin A, and hydroxylamine should be considered. HOCH AND RANDLES[15] observed that FCCP and 2,4-dinitrophenol caused an increase in measuring light fluorescence using whole cells in the presence of DCMU or dithionite. The experimental observations reported here for chloroplasts in the presence of DCMU are very similar to those of ref. 15, in which the effects of FCCP and 2,4-dinitrophenol were attributed to their inhibition of a proton gradient or high energy intermediate generated by System I cyclic electron flow. From measurements of fluorescence quenching by phenazine methosulfate and diaminodurol in DCMU-treated chloroplasts, it has been similarly concluded that the variable yield fluorescence can be quenched by high-energy intermediate generated by cyclic electron flow[21,22]. We would agree that proton permeability effects may be important in these experiments on measuring light fluorescence, since antimycin A has also been found to stimulate the dark decay of the proton pump in chloroplasts[23], and we have found that the weak-acid uncouplers S-13 and TTFB, which like 2,4-dinitrophenol and FCCP have been found to increase the conductance of artificial membrane systems[24,25], cause an increase in measuring light fluorescence yield and decay time. But we do not think that these compounds affect the fluorescence in the presence of DCMU by acting on

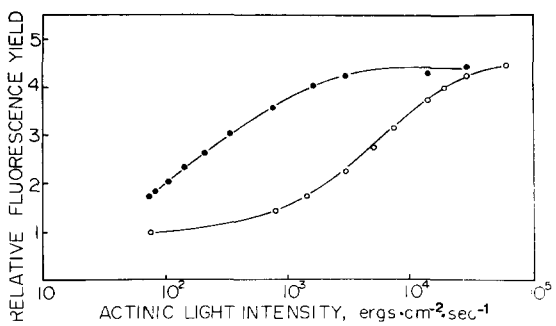


Fig. 11. Increase in fluorescence yield as a function of actinic light intensity in the presence (●) and absence (○) of DCMU. Fluorescence yield changes measured as in the first part of Figs. 1–3.

cyclic electron flow for two reasons: (1) it seems unlikely that cyclic electron flow in System I occurs to a significant extent under the aerobic conditions utilized for the chloroplast experiments and in the absence of any added cofactor; (2) we do not think that high-energy intermediate from System I cyclic electron flow is responsible for the fluorescence quenching by phenazine methosulfate in the presence of DCMU, since phenazine methosulfate in the presence of DCMU quenches the fluorescence of whole cells of Bishop's Scenedesmus mutant No. 8 as effectively as it quenches the fluorescence of the wild type (W. A. CRAMER, unpublished data). Mutant No. 8 is missing P_{700} and has no System I activity[26]. Thus, at the present time we feel that experiments reported here on changes of fluorescence yield are still most easily interpretable in terms of effects on the primary acceptor "Q". We feel that the ionic strength and in particular the concentration of divalent cations determine the maximum fluorescence yield[27,28], but that relative to this maximum the level is controlled by the redox state of "Q". Thus, we are led to explain the correlation between the changes in fluorescence parameters and the decrease in concentration of high-potential *b*-559 in terms of the *b*-559 being a secondary acceptor in the presence of DCMU. We note that except for 2,4-dinitrophenol, which we haven't tested, all of the agents mentioned above which affect proton uptake, proton conductance and the fluorescence parameters also increase the amount of low-potential *b*-559 which can be oxidized by Photosystem I (data not shown).

Implicit in the hypothesis that high-potential cytochrome *b*-559 can accept electrons from "Q" in the presence of DCMU at low light intensities is the assumption that the lower-potential form reached through the conversion cannot accept electrons from "Q" in the presence of DCMU. The low-potential *b*-559 is then either completely unlinked to the primary acceptor or it is on the main electron transport chain containing the DCMU block. We find that DCMU addition in the presence of actinic light generally causes an increase in the oxidation level of the cytochrome *b*-559, though the increase is small unless conditions in the dual wavelength measurement are such that an excess of low-potential *b*-559 has been formed. Thus, we tend to place the DCMU block on the System II side of low-potential cytochrome *b*-559, as shown in Fig. 10. This is consistent with most of the published data on the effect of DCMU on the photoresponse of the cytochrome *b*-559. There are reports, however, that at low DCMU concentrations[30] or in Tris-washed chloroplasts[31], DCMU may block on the oxidizing side of cytochrome *b*-559. These results lead to the impression that the DCMU block is very close to cytochrome *b*-559.

The ability of high-potential *b*-559 to affect the variable fluorescence of Photosystem II provides support for the proposal[8] that cytochrome *b*-559 is very close to System II and, in particular, closer than the plastoquinone pool (see Fig. 10). Cytochrome *b*-559 has recently been found to be greatly enriched in a partially purified Photosystem II particle[32]. But we cannot at this time propose a role for the high-potential *b*-559 other than as a pool from which low-potential *b*-559 can be withdrawn to participate in non-cyclic electron flow, the distribution between high- and low-potential forms performing a regulatory role[8]. It seems unlikely that the cyclic pathway around System II has any physiological function in the light reactions of photosynthesis since it is saturated at such low light intensities. Regarding other possibilities for the role of cytochrome *b*-559, Fig. 12 shows that high-potential *b*-559 cannot be on the pathway of water oxidation, perhaps involved in the extrac-

tion of the first electron of four from water, as recently suggested[11]. This latter hypothesis implies that converting an appreciable amount of the high-potential *b*-559 to a state with midpoint potential 200–300 mV more negative (where it is not reduced by hydroquinone at pH 7.8) should result in an inhibition of O_2 evolution. Fig. 12 shows that electron flow from water to methyl viologen is not inhibited at all over a period of at least 1 min by addition of concentrations of FCCP (1 μM) and hydroxylamine (0.6 mM) that would cause almost total conversion of the *b*-559 to a low-potential form within this time.

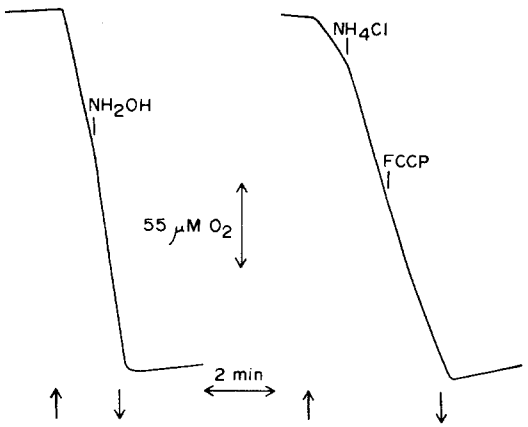


Fig. 12. Effect of hydroxylamine and FCCP on electron flow from water to methyl viologen. Oxygen uptake is measured as described in METHODS (*2*). In the first experiment 0.6 mM NH_2OH is added to the suspension already containing 3 mM NH_4Cl to give maximum initial uncoupling. In the second experiment 1 mM NH_4Cl and 2 μM FCCP are successively added.

Other effects of FCCP have been reported on the fluorescence of whole algal cells. In the presence of DCMU and high actinic light intensities, it has been found that high concentrations of FCCP (10 μM) lower the maximum fluorescence yield[33]. We have observed similar phenomena in chloroplasts, dependent upon incubation time, which we attribute to inhibitory effects of FCCP on the donor sites of Photosystem II[34,35]. It has also been found that FCCP and nigericin decrease the dark incubation time required for a maximum light induced fluorescence yield increase[36]. This dependence on dark incubation time is much smaller in chloroplasts, and we have not tried to determine any dependence on FCCP.

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