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Location of cytochrome *b*-559 between Photosystem II and Photosystem I in noncyclic electron transport

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Selective light-induced redox changes of cytochrome *b*-559 in relation to cytochrome *f* have been investigated at 20 °C in fresh spinach thylakoids under physiological conditions and in the presence of specific inhibitors and uncouplers. Light absorbed by PS II (650 nm) promotes the sequential reduction of cytochrome *b*-559 and cytochrome *f*, whereas light absorbed by PS I (720 nm) brings about the oxidation of cytochrome *f* and, by way of this, the oxidation of cytochrome *b*-559. Either DCMU or DBMIB completely blocks the reduction of the two cytochromes by PS II light, but not the oxidation of cytochrome *f* by PS I light. On the other hand, cyanide as well as polylysine inhibits almost totally the oxidation of both cytochromes by PS I light. From these and previous results, it is concluded that photoreduction of cytochrome *b*-559 by PS II occurs through the plastoquinone pool and that its photooxidation by PS I involves both plastocyanin and cytochrome *f*. It thus appears that cytochrome *b*-559 is located between plastoquinone and cytochrome *f* in noncyclic electron transport in chloroplasts.

Introduction

One of the least understood aspects of photosynthesis in chloroplasts is the light-induced transfer of electrons and translocation of protons coupled to noncyclic photophosphorylation [1–10]. In the scheme put forward by Arnon [11] in 1959, water was the initial electron donor and $NADP^+$ the final electron acceptor in the chloroplast electron transport chain driven by light-excited chlorophyll, but the redox properties of the intermediate cytochromes did not correspond with those of cytochromes *f* and b_6, the only ones known at the time to occur in chloroplasts [3]. To take this objection into account, Hill and Bendall [12] postulated in 1960 two light-driven steps, rather than one, that would cooperate in electron transfer through a cytochrome b_6–*f* sequence. In their hypothesis, however, water (possibly arising from phosphorylation) was split by the first light-driven redox reaction, both in bacterial chromatophores and chloroplasts [12]. In 1961, Losada et al. [13] separated two light reactions in noncyclic photophosphorylation of green plants: (a) the auxiliary reaction for photooxidation of water and (b) the bacterial type reaction [14] used both by photosynthetic bacteria and by green plants for photoreduction of $NADP^+$ and coupled photophosphorylation. Additional evidence was presented [13,15] that oxygen evolution in chloroplasts is catalyzed by a photosynthetic pigment different from that involved in the photoreduction of $NADP^+$. Simultaneously and independently, Duysens et al. [16] identified two pigment systems, which they called systems 1 and 2, in the red alga *Porphyridium cruentum* and observed that the oxidation of a *f*-like cytochrome was caused by excitation of system 1, and its reduction by excitation of system 2.

A second *b*-type cytochrome, namely, cytochrome b_3 or *b*-559, was identified in algal and higher plants chloroplasts by different approaches during the second half of the 1960's as a redox component closely linked to the reaction center of Photosystem II (PS II) by Lundegardh [17], Levine and Gorman [18], Boardman and Anderson [19] and Cramer and Butler [20]. This linkage was soon confirmed by Knaff and Arnon [21] through photooxidation of cytochrome *b*-559 by PS II at cryogenic temperatures and decisively validated in

Abbreviations: CCCP, carbonylcyanide 3-chlorophenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; PS I, Photosystem I; PS II, Photosystem II.

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1987 by Nanba and Satoh [22] by isolation of a PS II reaction center complex consisting of the D-1 and D-2 polypeptides and cytochrome *b*-559. Cramer's group [23] has lately presented solid evidence that cytochrome *b*-559 is a heme cross-linked heterodimer of about 14 kDa defined by the presence of a single histidine residue on each of the constituent polypeptides. During the last 10 years, our group [24–27] has thoroughly reexamined and characterized the redox and acid-base states and properties of cytochrome *b*-559 in both fresh spinach thylakoids and PS II particles. Comparison of the molecular properties of cytochrome *b*-559 purified from these two sources revealed [27], moreover, the existence of only one molecular species present in two interconvertible redox forms and tightly bound to PS II, no hint being found of the occurrence of a distinct low-potential form associated with Photosystem I (PS I) claimed by different researchers (cf. Refs. 27–30).

Although the biochemical, biophysical and biological molecular properties of PS II cytochrome *b*-559 are at the present stage relatively well established [23–28], there is much uncertainty and controversy regarding its location and function in green plants' photosynthetic electron transport [2,5,9,22,23], if such were definitely to be the role of this elusive and enigmatic cytochrome. The original model of Hill and Bendall [12] including the cytochrome b_6-*f* sequence was reconsidered when cytochrome *b*-559 was discovered and the authors suggested that it could be replaced by the cytochrome *b*-559–*f* sequence [3]. However, the initial model is again widely accepted, since Hurt and Hauska [31] isolated and characterized from spinach chloroplasts the cytochrome b_6-*f* complex as a plastoquinol-plastocyanin-oxidoreductase and Lam and Malkin [6] succeeded in reconstitution experiments with this complex, PS I and PS II of the chloroplast noncyclic electron transport pathway from water to $NADP^+$.

As will be discussed in the last section of this paper, present opinions about the role of cytochrome *b*-559 in photosynthesis are divergent and are in some cases mutually exclusive and only three of them, from the laboratories of Arnon [2], Butler [5] and our own group [9], depict cytochrome *b*-559 as being involved both in electron transfer and proton translocation in chloroplast noncyclic electron transport. Our proposal [9,25] is that cytochrome *b*-559 functions, representing a particular case of a general model [7,8], as a transducer of redox energy into acid-base energy by operating at two alternative midpoint potentials between the two photosystems and at two pK_a values between the extrathylakoid and intrathylakoid spaces.

In this paper we present additional evidence confirming that chloroplast cytochrome *b*-559 is located in the noncyclic electron transport chain between the reducing side of PS II and the oxidizing side of PS I, i.e., after plastoquinol and before cytochrome *f*.

Materials and Methods

Spinach thylakoids were prepared as previously described [25].

Light-induced redox changes of cytochromes *b*-559 and *f* were measured at 559 and 554 nm, versus 570 nm, respectively, in an Aminco DW-2a dual-wavelength spectrophotometer at 20 °C in a 3 ml cuvette containing the thylakoid suspension (66 μg chlorophyll · ml^{-1}) in 50 mM Tricine-KOH buffer (pH 7.5). Spectra in the α-band region of cytochromes were obtained by measuring the absorbance change every 2 nm, with the reference wavelength at 570 nm and fixing the measurement wavelength within the 540–570 nm range.

Selective illumination of either PS II or PS I was attained with Baird-Atomic narrow-band filters with maximal transmittance at 650 and 720 nm, respectively. An extramonochromator (Oriel 7240), adjusted at 565 nm, with a band width of 50 nm, was inserted between the spectrophotometer photomultiplier and the sample compartment to allow the passage of reference and measurement light.

Results

Illumination under physiological conditions of a fresh spinach thylakoid suspension at 20 °C with 650 nm light, which is selectively absorbed by PS II, promotes the reduction of cytochrome *b*-559 and cytochrome *f*, as evidenced by a marked increase in absorbance at 559–570 nm and to a significant extent at 554–570 nm, respectively (Fig. 1). Actually, when compared with that of PS II particles lacking cytochrome *f* (cf. Ref. 26), the spectrum of this change (inset of Fig. 1) is a broad one, extending along the α-band region of both cytochromes, but with a maximum at 559 nm. It should be noted that cytochrome *f* is primarily in its oxidized state in the fresh thylakoid preparations used, while cytochrome *b*-559 is about 1/3 in its oxidized, nonprotonated, low-potential form (cf. Refs. 25 and 26). After switching off PS II light, subsequent illumination of the sample with 720 nm light, which is preferentially absorbed by PS I, brings about mostly oxidation of cytochrome *f*, as also shown clearly in the inset of Fig. 1 by the shift of the spectrum towards the region of the α-band of this cytochrome. Control experiments proved that 720 nm light is much less efficient (about 30%) than 650 nm light in promoting photoreduction of cytochromes *b*-559 and *f* and that, after switching off 720 nm light, further illumination with 650 nm light induces additional reduction of the two cytochromes to the level proper to this type of light (data not shown).

The effect of a very low concentration of the protonophoric reagent CCCP on the photooxidation of cytochrome *b*-559 in a thylakoid preparation illuminated with PS I light is shown in Fig. 2. A thylakoid suspen-

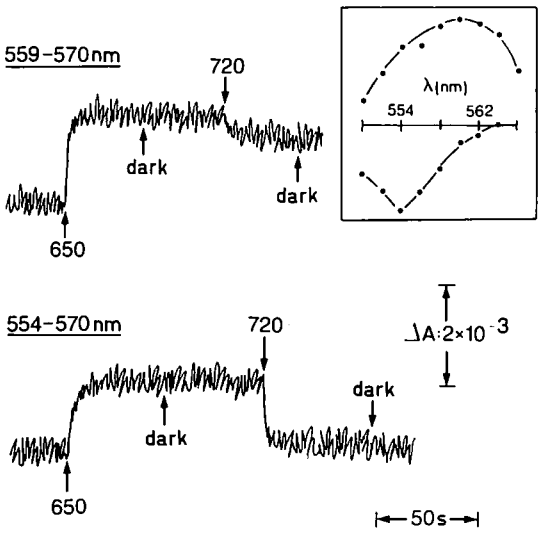

Fig. 1. Sequential photoreduction of cytochromes *b*-559 and *f* by PS II light and photooxidation of cytochrome *f* by PS I light in spinach chloroplasts. The absorbance redox changes at 559 and 554 nm induced by illumination at 20 °C of a fresh thylakoid suspension with light absorbed preferentially by PS II (650 nm) or PS I (720 nm) were determined as described in Materials and Methods. The spectra of the photoreduction of both cytochromes by 650 nm light and of the subsequent photooxidation of cytochrome *f* by 720 nm light are shown in the inset and were also determined as described in Materials and Methods.

sion was preilluminated with 650 nm light, in order to reduce cytochrome *b*-559 as well as cytochrome *f* (see also Fig. 1), and the PS II light was then turned off. Subsequently, the thylakoid preparation was illuminated with 720 nm light – which, as shown in Fig. 1, specifically induces photooxidation of cytochrome *f*

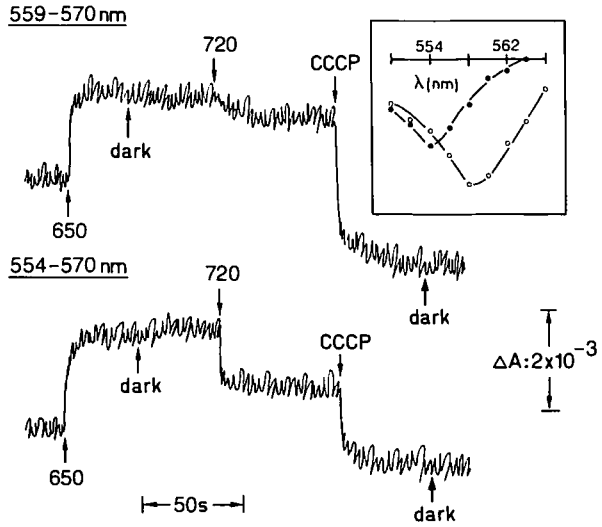

Fig. 2. Sequential photooxidation of cytochrome *f* and cytochrome *b*-559 by light absorbed by PS I: effect of CCCP in the light. The conditions were the same as in Fig. 1, except that 1 μM CCCP was added where indicated. The inset shows the spectra of cytochrome *f* photooxidation by 720 nm light in the absence of CCCP (●) and of the subsequent oxidation of cytochrome *b*-559 after addition of the uncoupler (○).

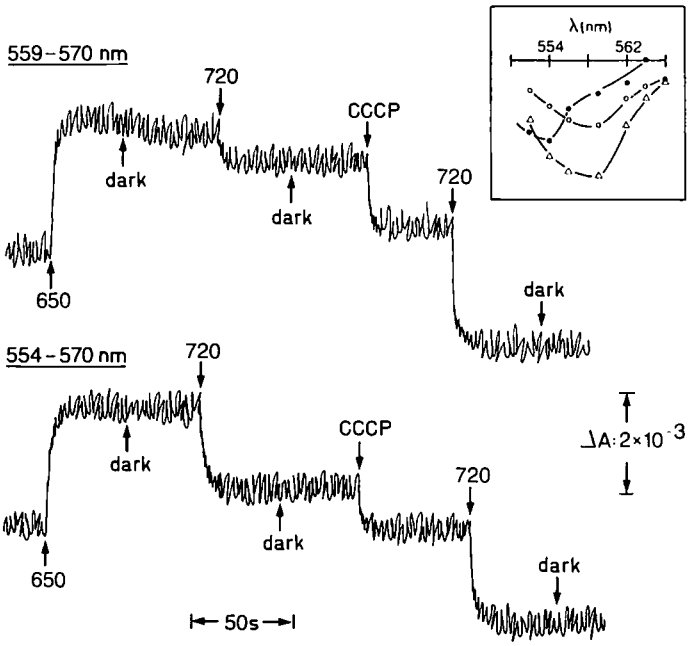

Fig. 3. Sequential photooxidation of cytochromes *f* and *b*-559 by light absorbed by PS I: effect of CCCP in the dark. The conditions were the same as in Fig. 2. The inset shows the spectra corresponding stepwise to the first photooxidation of cytochrome *f* by 720 nm light (●), to the oxidation of cytochrome *b*-559 by CCCP in the dark (○) and to further photooxidation of both cytochromes after the second illumination with 720 nm light (△).

– and 1 μM CCCP was added, keeping on the PS I light. Under these conditions, the addition of the uncoupler promotes a significant increase in the photooxidation brought about by PS I light, but in this case the extra-oxidation caused by CCCP is due almost exclusively to cytochrome *b*-559, as shown by the spectral analysis of the process (inset of Fig. 2). It should be emphasized that no or comparatively small absorbance changes were observed, respectively, when the uncoupler was added in the dark, prior to illumination with 720 nm light, or in the light, but under illumination with 650 nm light (data not shown). In other words, CCCP cannot directly oxidize per se cytochrome *b*-559 at the low concentration used, as it does at higher concentrations (cf. Refs. 24 and 26), but only by way of cytochrome *f* after its oxidation by PS I.

On the other hand, CCCP at micromolar concentration can also bring about oxidation of cytochrome *b*-559 in the dark if cytochrome *f* is previously photooxidized by illumination of the thylakoid preparation with PS I light. Fig. 3 shows the preparatory photoreduction of both cytochromes *b*-559 and *f* by 650 nm light and photooxidation of cytochrome *f* by 720 nm light (see also Figs. 1 and 2). Addition of CCCP in the dark to the thylakoid preparation pretreated in this manner promotes a significant oxidation which, as in the preceding experiment, is due mostly to cytochrome *b*-559 (inset of Fig. 3). Subsequent illumination with 720

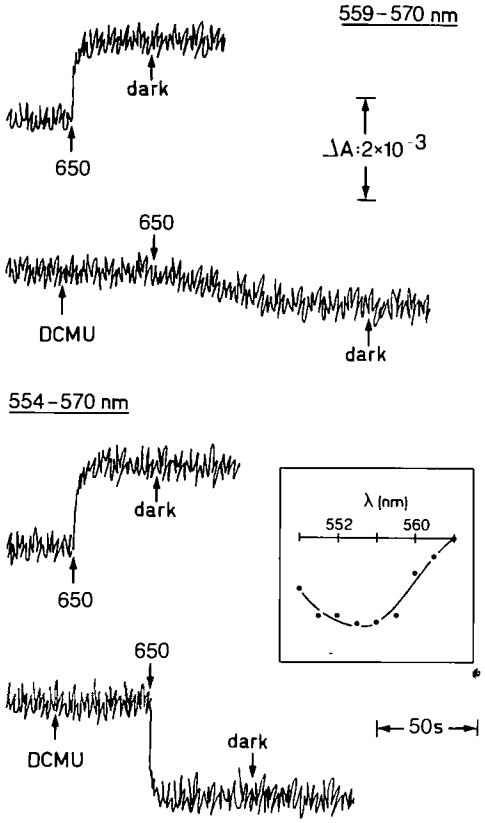

Fig. 4. Inhibition by DCMU of photoreduction of cytochromes *b*-559 and *f* by PS II, and photooxidation of cytochrome *f* by PS I in the presence of the inhibitor. The conditions were the same as in Fig. 1. Where indicated, 10 μM DCMU was added. The inset shows the spectrum of photooxidation of cytochrome *f* by 650 nm light in the presence of DCMU.

nm light further induces a notable oxidation, which as shown also in the inset of Fig. 3, involves not only cytochrome *f* but also cytochrome *b*-559. It is very important to realize that oxidation of cytochrome *b*-559 by CCCP in the dark can only occur after illumination with 720 nm light, but not with 650 nm light.

The electron transport inhibitor DCMU, which acts specifically at the reducing side of PS II at the plastoquinone level, completely blocks photoreduction of cytochromes *b*-559 and *f* by 650 nm light (Fig. 4). However, since this wavelength is, although selective for PS II, also partially active on PS I, an apparently paradoxical effect can be observed when 10 μM DCMU is blocking the photoreduction of the two cytochromes by PS II, namely, the specific photooxidation of cytochrome *f* by PS I (but excited by 650 nm light), ratified by the spectral change shown in the inset of Fig. 4. The effect of the plastoquinone antagonist DMBIB on the redox changes of cytochromes *b*-559 and *f* promoted by 650 nm light was also investigated in a parallel experiment. As shown in Fig. 5, 2 μM DBMIB behaves exactly as DCMU in impeding photoreduction of both cytochromes by PS II and in allowing photooxidation of cytochrome *f* by PS I (but excited by 650 nm light). The spectrum corresponding to this latter change is even more sharp and precise in the case of DBMIB (inset of Fig. 5).

An interesting phenomenon pertaining to the oxidation of cytochrome *b*-559 by PS I could, moreover, be observed, even in the absence of CCCP, when thylakoids were selectively illuminated in the presence of DCMU. Fig. 6 shows that illumination with 720 nm light of a thylakoid suspension preilluminated with 650 nm light and then treated with DCMU brings about a biphasic oxidation of the cytochromes. The spectral analysis of the process reveals beyond doubt that cytochrome *f* is the component being rapidly oxidized by 720 nm light, whereas cytochrome *b*-559 is the component undergoing slow oxidation by the same light. It seems obvious that, under conditions which impede photoreduction of the cytochromes by PS II, i.e., in the presence of DCMU, not only the fast photooxidation of cytochrome *f* by PS I can be observed, but also the slow photooxidation of cytochrome *b*-559 by this photosystem. The latter oxidation reaction is otherwise disguised in the absence of the inhibitor because it occurs simultaneously with the photoreduction of cytochrome *b*-559 by PS II (see also Figs. 1, 2 and 3). Final addition of CCCP results in

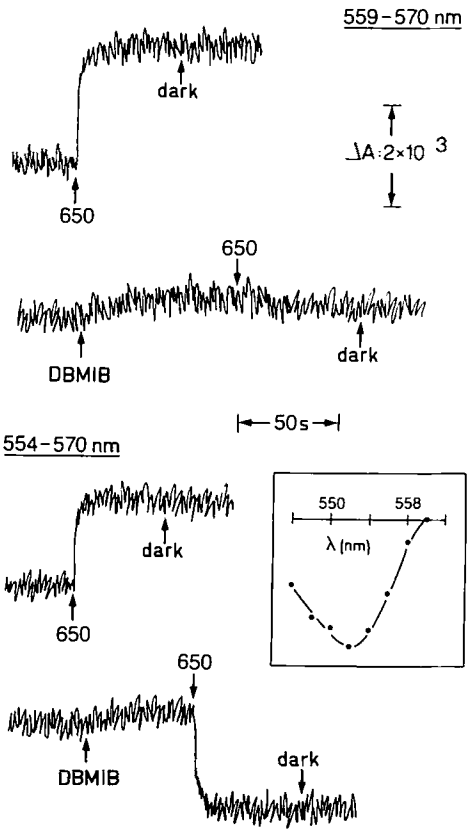

Fig. 5. Inhibition by DBMIB of photoreduction of cytochromes *b*-559 and *f* by PS II, and photooxidation of cytochrome *f* by PS I in the presence of the inhibitor. The conditions were the same as in Fig. 1. Where indicated, 2 μM DBMIB was added. The inset shows the spectrum of photooxidation of cytochrome *f* by 650 nm light in the presence of DBMIB.

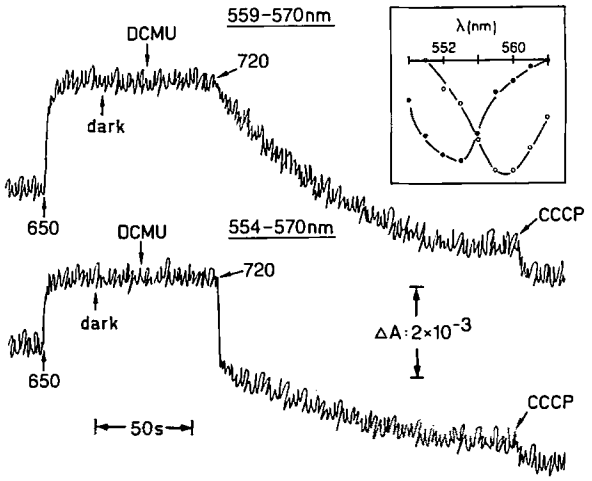

Fig. 6. Sequential photooxidation of cytochrome *f* and cytochrome *b*-559 by PS I in the presence of DCMU. The conditions were the same as in Fig. 1. Where indicated, 10 μM DCMU and 1 μM CCCP were added. The inset shows the spectra corresponding to the fast (●) and slow (○) components (cytochromes *f* and *b*-559, respectively) involved in photooxidation by 720 nm light after addition of DCMU.

no significant extra oxidation of either cytochrome. When DBMIB was used instead of DCMU, similar results were obtained concerning the photooxidation of cytochrome *f* by PS I, but not with respect to the oxidation of cytochrome *b*-559, which is indeed small, even in the presence of CCCP (data not shown).

The effect of cyanide, an inhibitor of photosynthetic electron transport at the plastocyanin level, on the light-induced absorbance changes of the α-band of cytochromes *b*-559 and *f* is shown in Fig. 7A and B, respectively. It can be seen that, compared with the control, preincubation of a thylakoid suspension with the inhibitor does not practically affect photoreduction of the cytochromes in response to illumination with 650 nm light. However, oxidation by 720 nm light is greatly lowered by cyanide, even in the presence of CCCP. The spectral analysis of the total change (Fig. 7C) shows that there is no shift in the spectrum after cyanide treatment, but only a significant and uniform decrease of absorbance at all the wavelengths concerned, thus suggesting that photooxidation of both cytochromes by PS I is similarly affected by the presence of cyanide.

The effect of polycation polylysine (an electron transport inhibitor, which, like cyanide, acts at the plastocyanin level) on the redox changes of cytochromes *b*-559 and *f* by 720 nm light was also investigated (Fig. 8). Here again, compared with the control, a clear inhibition of the photooxidation induced by PS I light is manifest, even after the addition of CCCP. Fig. 8C shows that, after oxidation by 720 nm light and in the presence of CCCP, the spectrum of this change involves both cytochromes either in the absence or in the presence of polylysine.

Discussion

There is wide agreement that, in chloroplasts, photosynthetic electron transport from water to $NADP^+$ in-

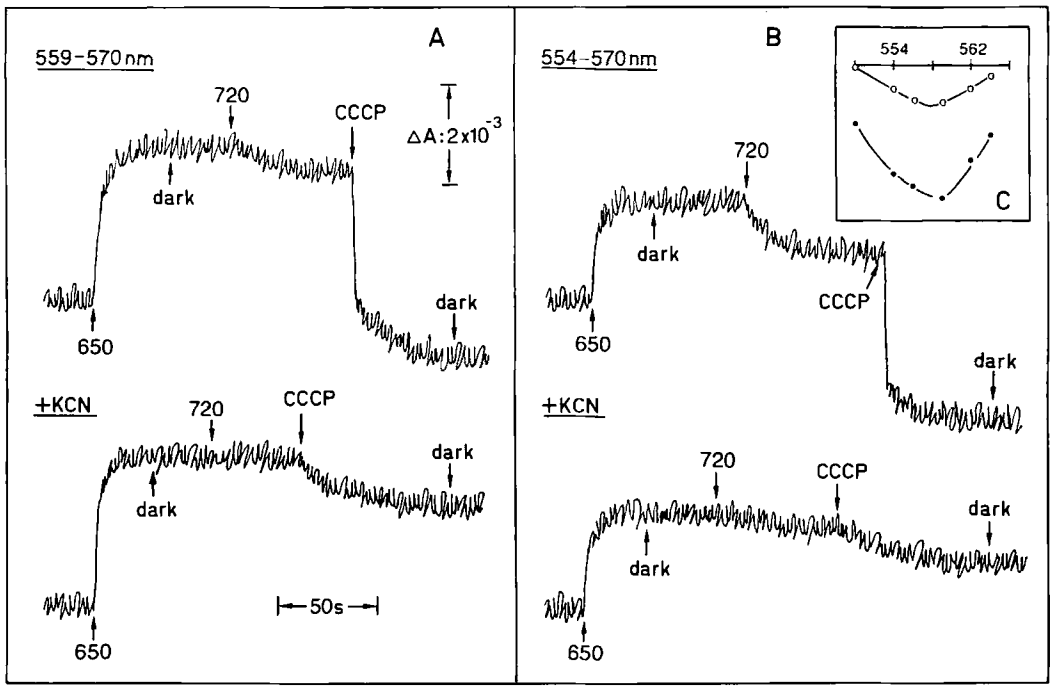

Fig. 7. Inhibition by potassium cyanide of photooxidation of cytochromes *f* and *b*-559 by PS I. The conditions were the same as in Fig. 1, but after incubation of the thylakoid suspension for 20 min at 20 °C with 20 mM potassium cyanide. Where indicated, 1 μM CCCP was added. The absorbance changes at 559 nm (A) and 554 nm (B) are compared in the control and cyanide-treated suspensions. The inset (C) shows the spectra of total photooxidation of both cytochromes by 720 nm light and CCCP in the absence (●) and in the presence (○) of potassium cyanide.

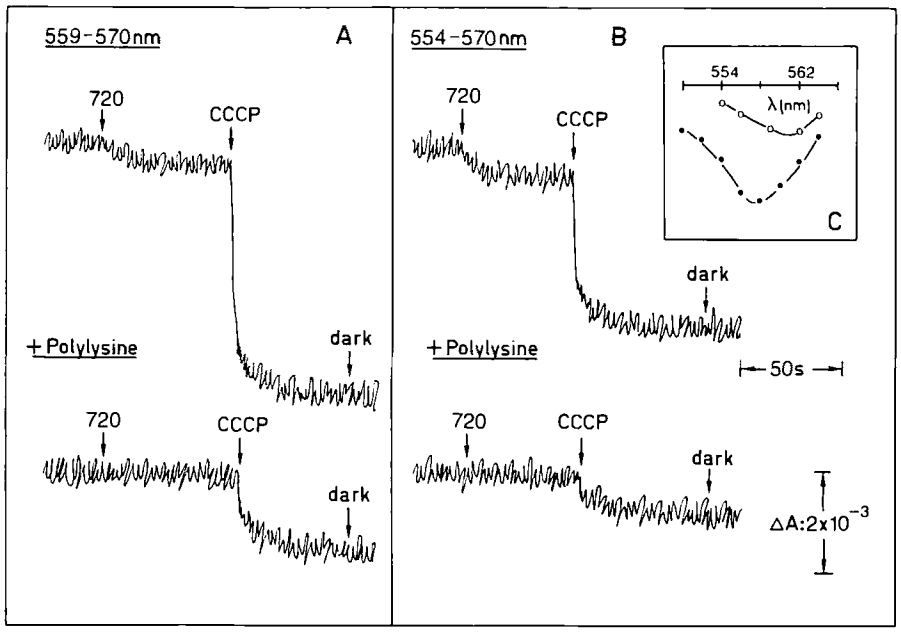

Fig. 8. Inhibition by polylysine of photooxidation of cytochromes *f* and *b*-559 by PS I. Polylysine (1 mg/ml) was added to a thylakoid suspension previously reduced with 650 nm light. The absorbance changes at 559 nm (A) and 554 nm (B) are compared in the control and polylysine-treated suspensions. Where indicated, 1 μM CCCP was added. Other conditions as in Fig. 1. The inset (C) shows the spectra of total photooxidation of both cytochromes by 720 nm light and CCCP in the absence (●) and in the presence (○) of polylysine.

volves PS II and PS I light reactions operating in series and connected by a chain of electron carriers which include plastoquinone on the reducing side of PS II and plastocyanin and cytochrome *f* on the oxidizing side of PS I. Electrons are sequentially transferred from P680, the photoreactive chlorophyll in the PS II reaction center, to Q_A, the single electron-carrying bound plastoquinone, and thence to Q_B, the bound secondary plastoquinone, which accepts two electrons and becomes protonated to form plastoquinol. On the other side, electrons are sequentially transferred from cytochrome *f*, by way of plastocyanin, to P-700, the photoreactive chlorophyll in the PS I reaction center. Nevertheless, there are still conflicting views about the inclusion of the *b*-type cytochromes in the noncyclic electron transport chain, as well as on the nature of the electron carrier(s) involved in proton translocation at the coupling site between the two photosystems [2–10,23,29,30].

Indeed, the precise role of cytochrome *b*-559 in photosynthetic electron transport has resisted elucidation for many years and some authorities in the field have even assigned unexpected functions in photosynthesis to it recently. To begin with, there is no consensus of opinion concerning the light-induced redox changes of cytochrome *b*-559 at room temperature. The earlier observations of Levine and Gorman [18] and Cramer and Butler [20] – and later of Ben-Hayyim and Avron [32] and Knaff [33] – appeared to be consistent with their proposal that cytochrome *b*-559 is on the electron transport pathway connecting the two photosystems, but shortly afterwards several workers failed to observe redox changes of cytochrome *b*-559 induced by light in untreated chloroplasts at room temperature [21,34–36]. Moreover, disagreement soon arose in the literature as to whether cytochrome *b*-559 is oxidized by either PS I or PS II or by both photosystems, especially from 1969 onward, when Knaff and Arnon [21] obtained unequivocal evidence that cytochrome *b*-559 is photooxidized at −189°C and that this photooxidation, which is inhibited by DCMU, can be induced only by 'short-wavelength' monochromatic light that activates the oxygen-evolving system in chloroplasts. This fact was explained [21] by assuming that at physiological temperatures the photooxidation of this cytochrome was immediately balanced by a reduction due to the flow of electrons from water. This interpretation was furthermore supported by the finding that photooxidation of cytochrome *b*-559 by 647 nm light occurs, even at room temperature, in Tris-treated chloroplasts, a treatment that inactivates PS II to the extent that, upon illumination, they cannot draw electrons from water. That cytochrome *b*-559 is closely associated with PS II and is, in fact, photooxidized via that system at low temperatures was soon confirmed by Floyd et al. [37], Boardman et al. [38], Bendall and Sofrová [39], and Erixon and Butler [40]. It must, however, be recognized that this photochemical reaction which occurs at liquid nitrogen temperature is not necessarily the primary photochemical event that takes place also at room temperature. The photooxidation of cytochrome *b*-559 by PS II at low temperature, or at room temperature in Tris-treated chloroplasts, may certainly be an artifact.

It must be emphasized that one of the reasons for the uncertainty concerning the role of cytochrome *b*-559 is the lack of light-induced redox changes observed at room temperature, not only in chloroplast preparations, but also in whole cells. For example, Amesz et al. [41] found no evidence for cytochrome *b* participation in cells of the red alga *Porphyridium cruentum* and of the green alga *Chlorella vulgaris*. Thus, in order to observe appreciable light-induced redox changes of cytochrome *b*-559 at room temperature it has generally been necessary to modify the chloroplasts in ways which may alter their operating conditions [2,30,34–36,42–46], so that the physiological significance of many of the experimental approaches has been open to question. In addition, there are, as mentioned above, divergent views as to whether the photooxidation of cytochrome *b*-559 is driven by PS I or PS II and even as to whether its role is in the main electron transport chain or in a cycle around PS II or on side pathways. For example, Heber et al. [30] have concluded from the redox behavior of high-potential cytochrome *b*-559 in the presence of CCCP that it is involved in cyclic electron transport around PS II. According to their hypothesis, photooxidation of high-potential cytochrome *b*-559, and its cyclic reduction, are physiological reactions under conditions such as high light intensities, when the oxidation capacity of the chlorophyll system exceeds that of the water-splitting machinery. Oxidation of cytochrome *b*-559 would then induce cyclic electron flow, preventing photooxidative reactions by the strong oxidant generated by PS II. Very recently, Thompson and Brudvig [47] have investigated the pathway of electron transfer between cytochrome *b*-559 and P-680 using EPR to show in frozen PS II membranes that the photooxidation of this cytochrome occurs via photooxidation of chlorophyll. They have suggested that cytochrome *b*-559 could serve to reduce the chlorophyll that is oxidized during photoinhibition. On the other hand, Cramer et al. [42] have formulated a thermodynamic mechanism for control of noncyclic electron transport in chloroplasts involving the high- and low-potential forms of cytochrome *b*-559.

Leaving aside several other proposals, two hypotheses from the laboratories of Arnon [2] and Butler [5] will be specially discussed here since, in common with our own, they depict cytochrome *b*-559 as being involved in both electron transfer and proton translocation in noncyclic electron transport. According to Butler [5], there is a significant correlation between high-potential cytochrome *b*-559 and the capacity of chloroplats to evolve oxygen. He postulated that cytochrome *b*-559 operates as a redox carrier between the two photosystems which binds protons on the oxidizing side of PS II and leads to proton release in the splitting of water. Arnon [2] has based his hypothesis on the finding [48] that DBMIB does not inhibit the photoreduction by PS II of high-potential cytochrome *b*-559, which is subsequently oxidized by PS II, and has proposed that the *b*-559 cycle around PS II functions as a redox-linked proton pump that operates in concert with the Rieske iron-sulfur–cytochrome *f* pathway in oxidizing plastoquinol. The concerted oxidation of plastoquinol by the Rieske iron-sulfur–cytochrome *f* pathway – which is sensitive to inhibition by DBMIB – and the *b*-559 cycle – which is insensitive to this inhibition – would provide for translocation of protons into the thylakoid lumen and for the transfer of electrons by noncyclic electron transport from water to plastocyanin (PC).

The results presented in this paper corroborate our previous proposal [9,25] that cytochrome *b*-559 is located between PS II and PS I, where it seems to operate as electron acceptor of plastoquinol (PQ) and as electron donor to cytochrome *f* in noncyclic electron transport, according to the following scheme:

$$\overset{h\nu}{\downarrow} \qquad\qquad \overset{h\nu}{\downarrow}$$
$$H_2O \rightarrow \text{PS II} \rightarrow \text{PQ} \rightarrow b\text{-559} \rightarrow f \rightarrow \text{PC} \rightarrow \text{PS I} \rightarrow \text{NADP}^+$$

As should be expected if a coupling site exists between cytochrome *b*-559 and *f*, we have obtained clear-cut evidence that cytochrome *b*-559 becomes specifically reduced by PS II light and cytochrome *f* becomes specifically oxidized by PS I light and that both cytochromes tend eventually to be reduced by PS II light and to be oxidized by PS I light, especially in the presence of the uncoupler CCCP. Moreover, it has been established that DCMU as well as DBMIB specifically block the reduction of cytochrome *b*-559 by PS II light, but not the oxidation of cytochrome *f* by PS I light, and that cyanide as well as polylysine inhibit oxidation of both cytochromes by PS I light.

The published effects of DCMU, DBMIB and CCCP on the light-induced absorbance changes of cytochrome *b*-559 – not only in chloroplasts but also in PS II particles (cf. Ref. 26) – are often contradictory. This might be explained by the various experimental conditions used in the different laboratories, e.g., Knaff and Arnon [49] and Boardman et al. [38] reported that DCMU inhibits the photooxidation of cytochrome *b*-559 at 77 K, McEvoy and Lynn [50] that this compound does not inhibit photoreduction of the cytochrome at room temperature, Ben-Hayyim [44] that photoreduction at room temperature by PS II can be detected only in the presence of DCMU, and Whitmarsh and Cramer [45] that DCMU inhibits the rate but not the extent of photoreduction of cytochrome *b*-559 by PS II. According to Cramer et al. [42] and Whitmarsh and Cramer [45], DBMIB accelerates the photoreduction of cytochrome *b*-559, but inhibits its oxidation by cytochrome *f*; in contrast, Knaff [33] reported that DBMIB com-

pletely blocks the photoreduction of cytochrome *b*-559 by PS II, but he did not observe any inhibition of either the rate or extent of its photooxidation by PS I.

To conclude this discussion, let us refer to some recent hypotheses that deserve special mention because they break with traditional views. Cramer et al. [23] have proposed a role for cytochrome *b*-559 as a mediator in the (re)assembly of the water-splitting enzyme either in developing chloroplasts or in thylakoids recovering from stress-induced damage, and Satoh [22] has suggested as a possible function for cytochrome *b*-559 that of a 'charge balancer' in the PS II core complex.

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