THE ROLE OF CYTOCHROME *f* IN FERREDOXIN-DEPENDENT ELECTRON-TRANSPORT REACTIONS IN SPINACH CHLOROPLASTS

Richard K. CHAIN
Department of Cell Physiology, University of California, Berkeley, CA 94720, USA

Received 22 June 1979

1. Introduction

Wide acceptance of the hypothesis of two light reactions in photosynthesis [1,2] has led to attempts to determine what electron carriers are located between the two photosystems (photosystem II and photosystem I). Based on the antagonistic effect of red versus far-red illumination and on the effect of 3-(3′,4′-dichlorophenol)-1,1-dimethylurea (DCMU) [2], cytochrome *f* has been proposed as one such carrier. In experiments with spinach chloroplasts, the cytochrome was demonstrated to function between water and the physiological electron acceptor ferredoxin-NADP [3]. The plastoquinone antagonist 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) [4,5] was found to function in a manner similar to DCMU in that both inhibitors prevented the reduction of the cytochrome by water but not its oxidation by photosystem I.

Although, based on data from kinetic experiments, others have suggested that cytochrome *f* may not be involved in the main noncyclic electron-transport chain [6], the data presented below will be interpreted in terms of cytochrome *f* being an obligate electron carrier between the two photosystems. As indicated above, the source of electrons for the reduction of cytochrome *f* under conditions of noncyclic electron transport can be either water (in the light) or (in the dark) more electronegative electron carriers, in the reduced form, between the primary electron acceptor for photosystem II and the cytochrome.

In addition to this ferredoxin-dependent noncyclic electron-transport system, reduced ferredoxin functions as an electron carrier in a cyclic electron-transport reaction [7] that was thought to involve cytochrome *f* [8–11]. Cyclic, in contrast to noncyclic electron transport, could be driven solely by photosystem I, and the associated ATP formation was inhibited (at low concentrations) by antimycin A [7] but not directly by DCMU. Cyclic photophosphorylation was also inhibited by DBMIB [7].

If cytochrome *f* is an electron carrier in both types of electron transport and inhibitors are used that are specific for the respective pathways (e.g., DCMU and antimycin A), it should be possible to demonstrate the reduction of the cytochrome from either water (as in noncyclic) or reduced ferredoxin (as in cyclic). (Cytochromes reviewed in [12,13].)

This communication reports the ferredoxin-dependent reduction of cytochrome *f* and the inhibition of that reduction by antimycin A. The results presented are consistent with the hypothesis that cytochrome *f* functions as an electron carrier common to both cyclic and noncyclic electron-transport reactions.

2. Materials and methods

Spinach chloroplast membranes were prepared as in [7]. To insure maximum activity, all experiments were completed within 30–45 min after preparation of the membranes. Cytochrome absorbance changes were followed in an Aminco DW-2 spectrophotometer operated in the dual-wavelength mode. Illumination was provided by filtering white light through three Corning 2-58 filters in conjunction with two Corning

4-96 filters that shielded the phototube. Red illumination was directed onto the surface of the cuvettes (2 mm) at an acute angle to the measuring beam from a mirror attached inside the sample compartment. The incident light intensity (8×10^4 ergs $.cm^{-2}$ $.s^{-1}$) was measured with a YSI-Kettering model 65 radiometer. The reference wavelength was 540 nm, with a slit width of 1.5 nm and an instrument time-constant of 300 ms.

3. Results

Figure 1 shows the oxidation–reduction pattern of cytochrome *f* when the terminal electron acceptor for noncyclic electron flow was ferredoxin-NADP. The cytochrome was oxidized during the first of two 5 s periods of illumination (fig.1A); during the following dark period it was partially reduced. Oxidation during the initial period of illumination was substantially more than during any of the ensuing periods of illumination. In fresh chloroplast membranes, the cytochrome was fully reduced before illumination (data not shown).

Although antimycin A (fig.1C) had no effect on the oxidation–reduction pattern relative to the control (fig.1A), the addition of DCMU (± antimycin A, see fig.1B,D) totally inhibited the reduction of cytochrome *f* in the dark after photooxidation.

When both the oxidized and reduced forms of NADP were added to the reaction mixture (fig.2), the oxidation–reduction pattern of cytochrome *f* exhibited pronounced changes with respect to the preceding figures: after initial oxidation in the light (fig.2A), the subsequent dark reduction was rapid and complete. In the presence of DCMU (fig.2B), the reduction curve of the cytochrome was biphasic, with the rapid phase reducing ~60% of the total cytochrome that had been oxidized in the light. The addition of antimycin A (fig.2C) again resulted in a

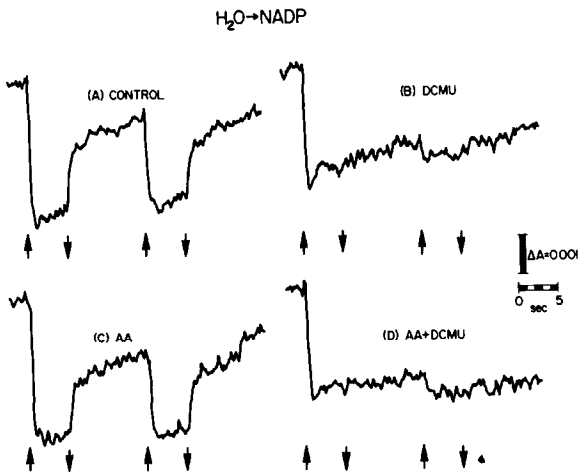

Fig.1. Oxidation–reduction pattern of cytochrome *f* (554–540 nm) under conditions of noncyclic electron flow to ferredoxin-NADP. The reaction mixture (final vol. 1.0 ml) consisted of chloroplasts (500 μg/ml) and the following (μmol): Tricine–KOH buffer (pH 8.35), 100; $MgCl_2$, 5; ADP, 3.5; KPO_4, 2; NADP, 1.0; and ferredoxin, 0.05. Where indicated, DCMU (25 μM) and antimycin A (37 μM) were added. Upward arrows, light on; downward arrows, light off. Downward deflection indicates oxidation of cytochrome *f*.

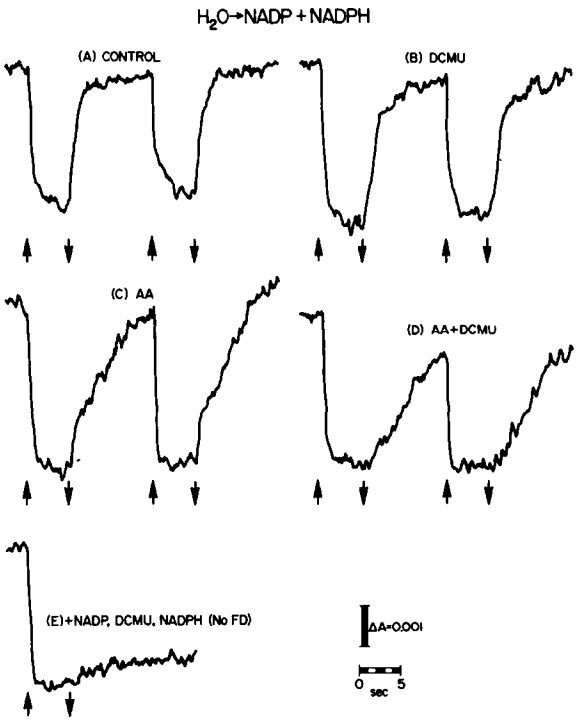

Fig.2. Oxidation–reduction pattern of cytochrome *f* (554–540 nm) under conditions of both cyclic and noncyclic electron flow. Conditions and concentration of inhibitors were as described in fig.1 except that NADPH (2.5 mM) was included in the reaction mixture.

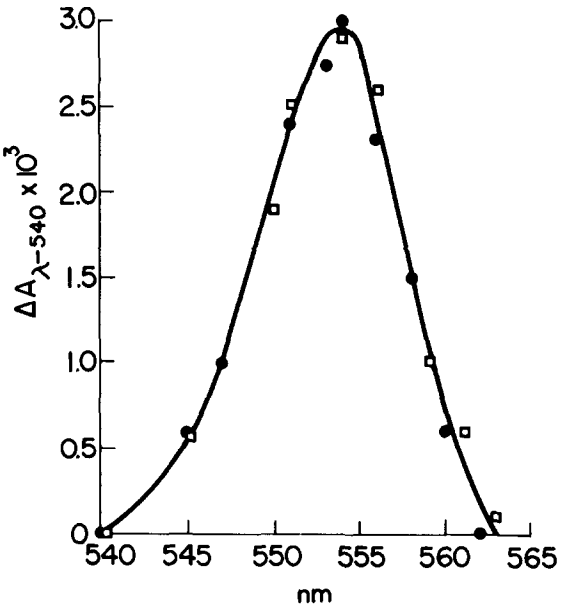

Fig.3. Spectrum of dark absorbance (554–540 nm) increase after illumination under conditions of cyclic electron flow. Conditions and concentration of DCMU as in fig.2B except that NADPH, (□) 2.5 mM, or (•) 5.0 mM, was added.

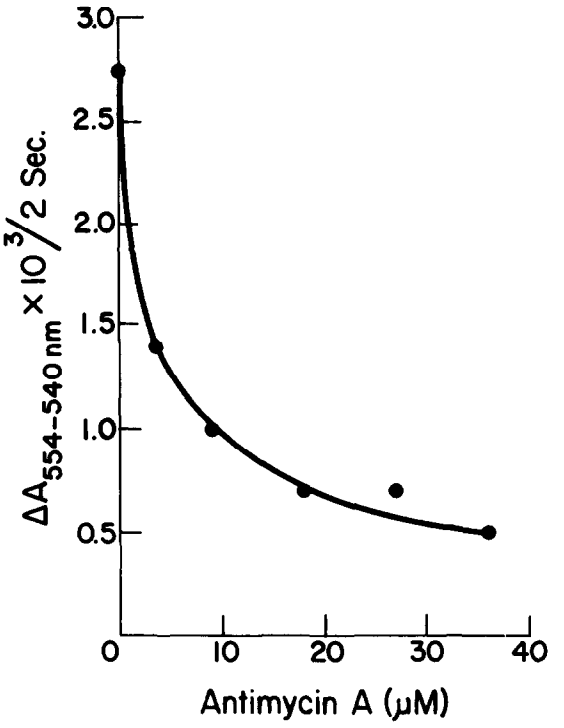

Fig.4. Inhibition of cytochrome *f* reduction (554–540 nm) by antimycin A. Conditions and concentration of DCMU and NADPH as in fig.2B with antimycin A added as indicated.

biphasic curve, but the rapid phase accounted for only ~30% of the total reduction. In the presence of both antimycin A and DCMU (fig.2D), reduction was slow and not complete within the time-span of the experiment. In fig.2E is depicted an absolute requirement of ferredoxin for the reduction of cytochrome *f* under conditions where the flow of electrons from water is excluded from the system.

The spectrum of the rapid, dark absorbance increase (fig.2B) was plotted as a function of wavelength in fig.3. This spectrum, with an absorption maximum at 554 nm, is characteristic of cytochrome *f*. Little or no cytochrome b_{559} or b_6 could be detected in the spectrum under the conditions used in fig.2B. Chemical (oxidized minus reduced) difference spectra (data not shown) revealed that ~83% of the hydroquinone-reducible cytochrome *f* was reduced under the conditions of fig.2B. The ratio of cytochrome *f* to chlorophyll was 1:570.

The inhibitory effect of antimycin A on the ferredoxin-dependent reduction of cytochrome *f* in the presence of NADPH and DCMU is shown in fig.4 (conditions as in fig.2B). The rapid phase of cytochrome *f* reduction was inhibited 50% at 3.6 μM antimycin A. In these experiments, it was not possible to ascertain what effect DBMIB has on the dark reduction of cytochrome *f*. Other experiments in this laboratory have shown that DBMIB can function as an effective reductant of some of the bound electron carriers in chloroplasts, including cytochrome *f* [14].

4. Discussion

Figure 5 represents an abbreviated model of the two pathways by which cytochrome *f* may be reduced and shows the inhibitors that could be expected to affect this reduction. With H_2O as the only source of electrons for the reduction of cytochrome *f*, DCMU should have a pronounced effect on reduction in the dark after illumination. Similar results could be expected if reduced ferredoxin was the sole source of electrons for the reduction of cytochrome *f* and this source was interrupted by antimycin A.

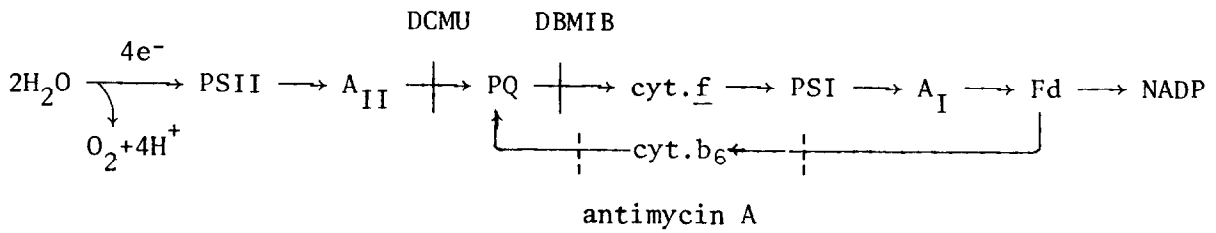

Fig.5. Proposed pathways of interaction of some of the electron carriers in cyclic and noncyclic electron transport. Fd, ferredoxin; cyt. b_6, cytochrome b_6; PQ, plastoquinone; cyt. *f*, cytochrome *f*; PSI, reaction-center chlorophyll for photosystem I; PSII, reaction-center chlorophyll for photosystem II; A_{II}, primary electron acceptor for photosystem II; A_I, primary electron acceptor for photosystem I.

The results presented in fig.1 illustrate that, when chloroplast membranes were illuminated with red light under conditions of coupled noncyclic electron flow, cytochrome *f* could be only partially reduced in the ensuing dark period. In experiments with DCMU it was shown that electrons flowing through the system in the light were from water. The incomplete reduction of the cytochrome in the dark suggested that the number of electrons in the plastoquinone pool was not sufficient to fully reduce the cytochrome in the dark [15].

The results typified by fig.2 demonstrate that reduced ferredoxin can function as an electron donor for the reduction of cytochrome *f*. When DCMU was added (fig.2B), although it totally inhibited all electron flow from water (see fig.1B), it did not substantially affect the reduction of the cytochrome. The possibility must be considered that the reduction of cytochrome *f* (+350 mV) by ferredoxin (–420 mV) was simply a chemical reduction of a more electropositive bound electron carrier by an electronegative soluble electron donor. The results shown in fig.2D clearly indicate that, although inhibition of the reduction of the cytochrome was not complete, a substantial inhibition of the rapid phase of reduction had occurred. Inhibition of electron flow in this system is consistent with inhibition of ATP formation in steady-state illumination under conditions of cyclic electron flow [7]. Inhibition of cytochrome *f* reduction by antimycin A argues against a nonspecific chemical reduction of the cytochrome and supports the view that cytochrome *f* functions as an electron carrier in cyclic electron transport and phosphorylation.

The loss of the rapid phase of reduction in the presence of antimycin A (fig.2C) after the addition of DCMU (fig.2D) suggests that water was the source of electrons for this change. The slow reduction of the cytochrome shown in fig.2D was interpreted as resulting from a 'leak' of electrons through the antimycin A inhibition site.

The results presented in fig.2 support the hypothesis that the reduction of cytochrome *f* by ferredoxin involves the main pathway of cyclic electron transport and that the reduction of cytochrome *f* by water represents the main pathway of noncyclic electron transport and phosphorylation, i.e., cytochrome *f* is apparently an electron carrier common to both types of electron flow. Recent experiments in this laboratory have demonstrated that other bound electron carriers could be reduced by reduced ferredoxin [7].

The reduction of C-550 [16] in the dark was found to be a ferredoxin-dependent reaction, and the electron flow between reduced ferredoxin and C-550 was inhibited by DCMU and antimycin A but not by DBMIB [17]. Cytochrome b_6 was also reduced by reduced ferredoxin [17]. These observations would suggest that electrons from reduced ferredoxin enter the noncyclic electron-transport chain at a point between the sites of inhibition of DCMU and DBMIB [17]. The involvement of cytochrome b_6 is assumed on the basis of the strong antimycin A sensitivity of the reduction of cytochrome *f* and of the inhibition of cytochrome b_6 photooxidation by DBMIB [8].

Cytochrome *f*, cytochrome b_6, and C-550 are not the only electron carriers that are reduced by ferredoxin in an antimycin A-sensitive reaction. The 'Rieske' iron–sulfur center, plastocyanin, and *P*-700 all behave in a manner similar to cytochrome *f*

(R. Malkin, in preparation).

Integration of these observations with our previous knowledge of the interaction of cyclic and noncyclic electron flow and concurrent photophosphorylation [7,18] in steady-state light supports in general the sequence of electron carriers depicted in fig.5.

Acknowledgements

This work was supported in part by a grant from the National Institutes of Health to Richard Malkin. I would like to thank Dr Malkin for many helpful discussions during the preparation of this manuscript. DBMIB was a gift from Professor A. Trebst.

References

[1] Duysens, L. N. M., Amesz, J. and Kamp, B. M. (1961) Nature 190, 510–511.
[2] Duysens, L. N. M. and Amesz, J. (1962) Biochim. Biophys. Acta 64, 243–260.
[3] Avron, M. and Chance, B. (1965) in: Currents in Photosynthesis (Thomas, J. B. and Goedheer, J. C. eds) pp. 455–463, Donker, Rotterdam.
[4] Trebst, A. (1974) Annu. Rev. Plant Physiol. 25, 423–458.
[5] Böhme, H. and Cramer, W. A. (1971) FEBS Lett. 15, 349–351.
[6] Haehnel, W. (1972) Biochim. Biophys. Acta 305, 618–631.
[7] Arnon, D. I. and Chain, R. K. (1975) Proc. Natl. Acad. Sci. USA 72, 4961–4965.
[8] Böhme, H. (1976) Z. Naturforsch. 31c, 68–77.
[9] Böhme, H. and Cramer, W. A. (1972) Biochim. Biophys. Acta 283, 302–315.
[10] Knaff, D. B. (1977) Arch. Biochem. Biophys. 182, 540–545.
[11] Slovacek, R. E. and Hind, G. (1978) Biochem. Biophys. Res. Commun. 84, 901–906.
[12] Cramer, W. A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133–172.
[13] Knaff, D. B. (1978) Coord. Chem. Rev. 26, 47–70.
[14] Chain, R. K. and Malkin, R. (1979) Arch. Biochem. Biophys. in press.
[15] Böhme, H. and Cramer, W. A. (1972) Biochemistry 11, 1155–1160.
[16] Knaff, D. B. and Arnon, D. I. (1969) Proc. Natl. Acad. Sci. USA 63, 963–969.
[17] Arnon, D. I. and Chain, R. K. (1979) FEBS Lett. 102, 133–138.
[18] Chain, R. K. and Arnon, D. I. (1977) Proc. Natl. Acad. Sci. USA 74, 3377–3381.