PHOTOSYNTHETIC ELECTRON TRANSPORT IN THE BUNDLE SHEATH OF MAIZE

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1. Introduction

Initial investigations into the mechanism of C4 photosynthesis showed that in species such as maize, CO_2 fixed in the mesophyll cells is transported in the form of malate to the bundle sheath cells, where its decarboxylation is catalyzed in the chloroplasts by $NADP^+$-dependent malic enzyme [1]. The presence of agranal chloroplasts in the bundle sheath of these species [2] and the functional deficiency of photosystem II activity [1,3,4] led to the view that the NADPH formed during the decarboxylation of malate is essential for reduction of part of the glycerate-1,3-bisphosphate formed in the reductive pentose phosphate pathway. The marked stimulation of photosynthesis which malate causes in isolated bundle sheath tissue is consistent with this view [3,5]. Although the extent of the deficiency in photosystem II has since been questioned [6,7], recent work with bundle sheath strands having photosynthesis rates equal to those of the parent tissue implied that photosynthetic O_2 evolution (and uptake) with CO_2 as the acceptor is limited and that the high ATP demand for CO_2 fixation is largely met by cyclic photophosphorylation mediated by photosystem I rather than via noncyclic or pseudocyclic photophosphorylation [5]. Cyclic photophosphorylation requires activation by electron flow from a suitable reductant, which establishes appropriate redox poise in the electron carriers. In this letter, flash spectrophotometric studies are described which indicate that photosystem II activity is inadequate to poise cyclic electron flow in bundle sheaths of maize, and that malate decarboxylation supplies the necessary electrons.

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2. Experimental

The light-induced absorbance change at 518 nm (P518) in chloroplasts indicates the formation of an electric field across the thylakoid membrane [8]. In intact chloroplasts it is characterised by a fast rise (occurring in ns) followed, under conditions favouring cyclic electron flow, by a slow (ms) rise. The slow phase is thought to reflect an electrogenic step associated with cyclic electron transport because it bears a specific relation to other aspects of cyclic activity such as cytochrome turnover, photophosphorylation and ΔpH, and because of its sensitivity to antimycin A and redox poise [9–13].

Zea mays (Pioneer hybrid 3780) was grown in a naturally illuminated greenhouse for 2–3 weeks. Strands of bundle sheath cells were isolated from fully expanded leaves essentially as in [5] except for minor variations in blending times and the digestion medium which was modified to contain 2% (w/v) cellulase and 0.3% (w/v) pectinase (as Cellulysin and Macerase from Calbiochem) and from which glucose 6-phosphate and ribose 5-phosphate were omitted. Microscopic examination of isolated strands showed them to be completely free of mesophyll contamination. Absorption changes at 518 nm induced by single turnover flashes were recorded and averaged as in [11].

3. Results

Fig.1 shows the flash-induced P518 response observed in bundle sheath strands of maize. The spectrum of the flash-induced response (not shown) was similar to that reported for spinach chloroplasts [9,11], with a maximum near 518 nm, but with a considerably smaller amplitude relative to the cytochrome changes

or to the chlorophyll content. Without additions, the fast phase of P518 was small and there was little evidence of a slow component in the absorption increase (fig.1a). Addition of bicarbonate (fig.1b) had no effect but when ribose 5-phosphate (a primer of the carbon reduction cycle) was added, the amplitude of the fast phase increased (showing some turnover of the reaction centres) but without significant increase in the slow phase (fig.1c). Only addition of malate elicited a pronounced slow rise (fig.1d), indicating efficient operation of the ATP-generating cycle [9–13]. When nigericin was added, the amplitude of the fast phase increased slightly and the rise time of the slow component diminished (fig.1e) as would be expected if ΔpH were controlling photosynthesis. Lack of photosystem II involvement was shown by the insensitivity of these changes to 10 μM DCMU (fig.1f).

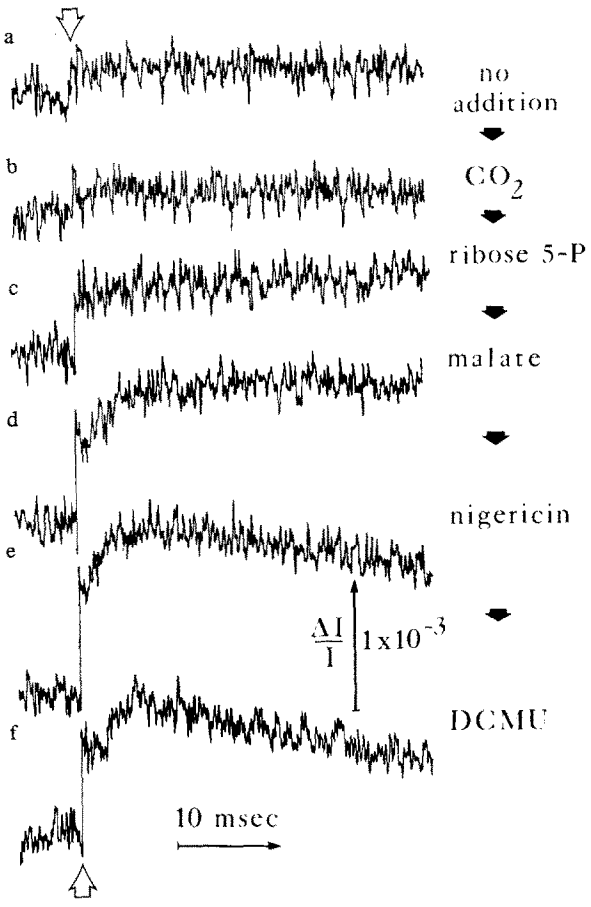

Fig.1. Flash-induced absorbance change at 518 nm in bundle sheath strands. The traces represent the average of 256 records at a flash frequency of 2 Hz. The reaction mixture contained in 1.5 ml: 0.36 M sorbitol, 3 mM $MgCl_2$, 1.5 mM K_2HPO_4, 3.8 mM K_2SO_4, 7.5 mM Tricine–KOH (pH 8.2), 15% (w/v) Dextran T-110 (Pharmacia) and bundle sheath strands (55 μg chl). Cumulative additions: (a) no additions; (b), (a) + 10 mM $NaHCO_3$; (c), (b) + 10 mM ribose 5-phosphate; (d), (c) + 10 mM malate; (e), (d) + 2.7 μM nigericin and (f), (e) + 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Open arrows indicate firing of the flash; solid arrows show the sequence of additions. Typical rates of photosynthetic CO_2 fixation measured at 20°C in the same medium, but in the absence of Dextran T-110, were (b) 9, (c) 34, (d) 109, μmol $Co_2 . h^{-1} . mg\ chl^{-1}$. These rates were not corrected for $^{14}CO_2$ dilution by malate decarboxylation. Dextran T-110, which was added to prevent settling of the strands in the cuvette, lowered these rates by ~20%.

Fig.2. Flash-induced turnovers of cytochrome *f* and b_{563}, shown in fig.2. Little activity was seen in the presence of bicarbonate or even ribose 5-phosphate (fig.2a,b) but substantial turnover of both cytochromes appeared following the addition of malate (fig.2c). The turnovers of both were accelerated upon collapsing the H^+ gradient with nigericin (fig.2d).

4. Conclusions

Photosystem II is essentially inactive in maize bundle sheath tissue, for in the absence of malate there is insufficient electron flow to prevent overoxidation of cycle intermediates and there is no sensitivity of the P518 absorbance change to DCMU. In the presence of malate, such photosystem II activity as exists might be further decreased by electron flow from NADPH leading to closure of photosystem II traps [14–16]. Recent independent estimate have confirmed that photosystem II activity in bundle sheath is low [17–18].

A further conclusion is that in *Zea*, malate synthesized in mesophyll tissue is presumably responsible for the redox poising and initiation of cyclic electron flow in the bundle sheath. The importance of correct redox poise in cyclic flow has been emphasized in work on C3 plants [9–16,19–22]: excessive photosystem II activity can lead to over-reduction of the carriers mediating cyclic electron flow, whereas inadequate photosystem II activity can lead to their over-oxidation. Removal of electrons from ferredoxin by O_2 or other electron acceptors has also been shown to influence cycle turnover [20,22]. In bundle sheath strands, the deficiency in photosystem II activity would be

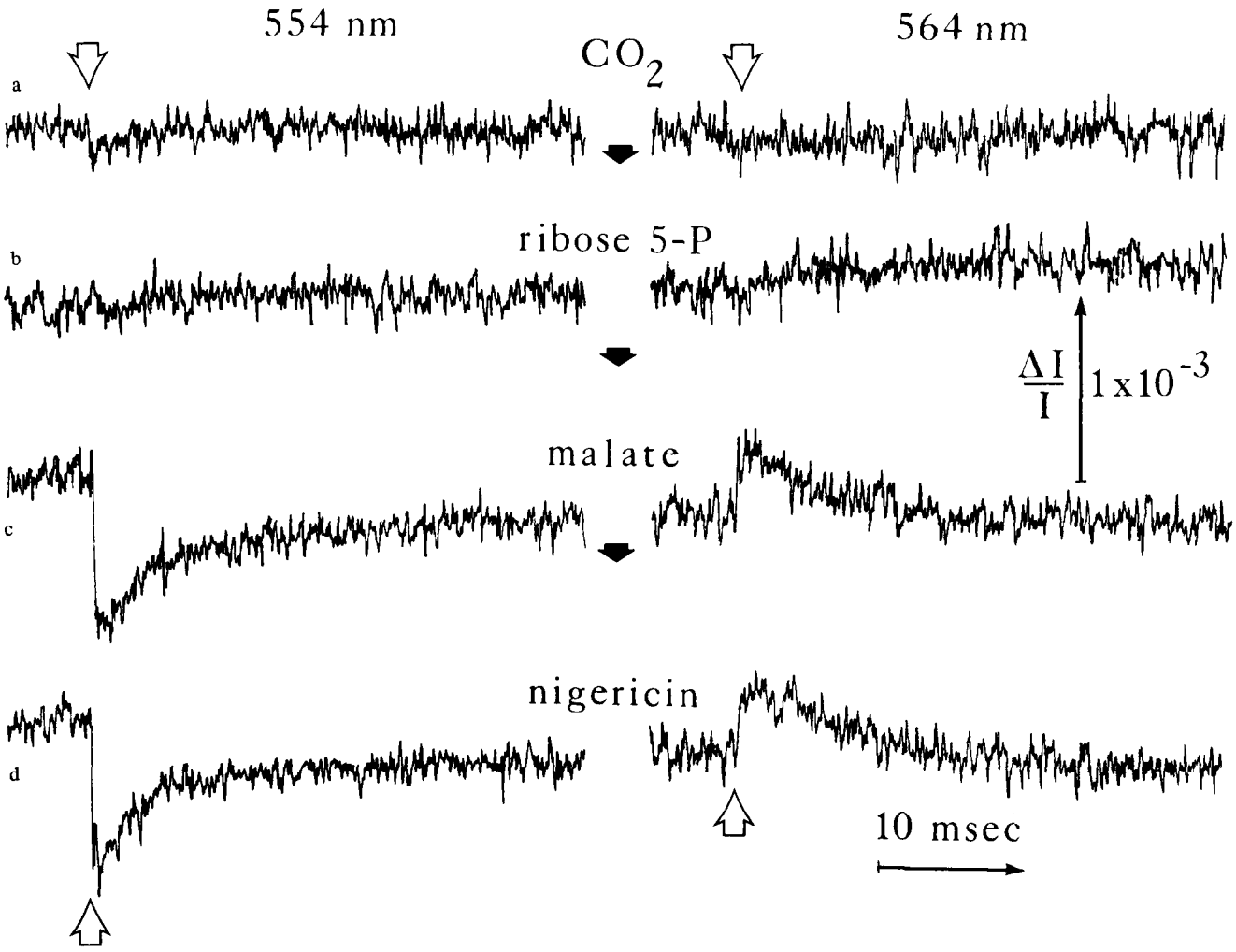

Fig.2. Flash-induced cytochrome *f* and cytochrome b_{563} turnovers in bundle sheath strands. Absorbance changes at 554 nm (cytochrome *f*) and 564 nm (cytochrome b_{563}) induced by single turnover flashes were recorded and averaged as in [11]. The traces represent the average of 512 records at a flash frequency of 2 Hz. Addition of sufficient valinomycin to collapse P518 within the time scale of these measurements did not cause significant changes in the sizes of the signals at 554 nm and 564 nm, showing that the 'tail' of the P518 response did not interfere with cytochrome measurements. Conditions as for fig.1b–e.

expected to lead to over-oxidation and hence a low rate of turnover of cyclic electron flow, particularly in the presence of electron acceptors or of O_2. The simplest explanation of the effect of malate is that its decarboxylation leads to an increase in the NADPH/$NADP^+$ ratio and that NADPH supplies electrons to the cyclic pathway. NADPH rather than photosystem II would thus act as the physiological poising agent in the bundle sheath. In similar experiments with spinach and with maize mesophyll (not shown) malate failed to poise DCMU-inhibited chloroplasts, presumably because of the absence of malic enzyme and because the equilibrium between malate and oxaloacetate, catalyzed by malate dehydrogenase, strongly favours malate formation.

In C3 photosynthesis, electron transport is now known to activate the carbon reduction pathway by one or more mechanisms which are currently being studied [23,24]. In the bundle sheath of *Zea*, a higher order of control appears to reside at the level of malate decarboxylation, which must first activate electron transport.

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