*Biochimica et Biophysica Acta*, 592 (1980) 495—505


BBA 47930

# RELATIVE ACTIVITIES OF LINEAR AND CYCLIC ELECTRON FLOWS DURING CHLOROPLAST $CO_2$-FIXATION

RUDOLF E. SLOVACEK, DAVID CROWTHER and GEOFFREY HIND

*Biology Department, Brookhaven National Laboratory, Upton, NY 11973 (U.S.A.)*

(Received March 27th, 1980)

*Key words: Photosynthesis; Electron transport; Cytochrome b-563; Cytochrome f; Photophosphorylation; $CO_2$ fixation*

## Summary

Evolution of oxygen and turnover of cytochromes *b*-563 and *f* were measured upon illumination of isolated intact spinach chloroplasts with a series of flashes. The flash yield of cytochrome *f* oxidation approximated the sum of the yields of cytochrome *b*-563 reduction and electron transfer through Photosystem II, regardless of whether $HCO_3^-$, 3-phosphoglycerate or $O_2$ served as the terminal electron acceptor. No absorbance contribution from cytochrome *b*-559 was discerned within the time range studied. Some pseudocyclic electron flow occurred when both $HCO_3^-$ and 3-phosphoglycerate were omitted, and possibly also during induction of photosynthesis; however, the flash yield data suggest that $O_2$ is not reduced at a significant rate during steady state photosynthesis. The maximum rate of cytochrome *f* turnover (1000 μequiv./mg chlorophyll per h) was adequate to support the highest rates of photosynthesis observed in isolated chloroplasts.

These results agree with the concept that cytochrome *f* is a component both of the linear and cyclic pathways whereas cytochrome *b*-563 functions only in the cyclic pathway. $NH_4Cl$ decreased the half time of cytochrome *b*-563 oxidation from 11.6 to 8.2 ms and decreased the half time of cytochrome *f* reduction from 7.2 to 2.8 ms. The cyclic and linear pathways thus seem to be jointly regulated by a transthylakoid $H^+$ gradient through a common control point on the reducing side of cytochrome *f*. Cyclic turnover also increased during the induction phase of photosynthesis, when linear throughput is limited by the rate of utilization of NADPH. The slow rise in the *P*-518 transient correlated with increased cyclic activity under the above conditions.

It is proposed that flexibility in the utilization of linear and cyclic pathways

---

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; *P*-700, reaction center for Photosytem I.

allows the chloroplast to generate ATP and NADPH in ratios appropriate to varying needs.

## Introduction

In chloroplasts, cyclic electron transport driven by Photosystem I is coupled to phosphorylation and is known to involve ferredoxin [1], cytochrome *b*-563 [2], and the sequence of electron carriers between plastoquinone and *P*-700 [3]. Recent spectroscopic studies [4] provided kinetic evidence that cytochrome *b*-563 and cytochrome *f* function on the reducing and oxidizing sides of a coupling site respectively, and showed that antimycin inhibits cytochrome *b*-563 oxidation. The involvement of cyclic phosphorylation in meeting some of the energy demand of photosynthesis was previously demonstrated by the sensitivities to antimycin of phosphorylation [1], $CO_2$ fixation [5,6], the transthylakoid proton gradient [7], the electrochromic effect [4], and chlorophyll fluorescence quenching [7].

Earlier studies of the cyclic system in isolated chloroplasts rested on indirect measures of electron flow such as phosphorylation [1] or some related parameter [6,8—10] following inhibition of linear and pseudocyclic [44] electron flows. The rate of cyclic phosphorylation was shown to depend on redox poise, which could be experimentally controlled by varying the oxygen tension [1,7,10] or by partially inhibiting Photosystem II activity (with DCMU [1,7] or far-red illumination [1,10]). A more physiological mechanism of redox poising by the closure of Photosystem II traps with NADPH was suggested from the work of Mills et al. [11] and Arnon and Chain [1,45]. However, investigation of this mechanism and the interrelationship between linear and cyclic electron flow during $CO_2$ fixation has been limited by inability to measure cyclic turnover directly.

In this study, chloroplasts were illuminated with repetitive flashes, and oxygen evolution rates, cytochrome *b*-563 and cytochrome *f* turnovers were measured during the reduction of 3-phosphoglycerate or $CO_2$. The influence of uncouplers and other conditions on cyclic turnover and Photosystem II activity was examined.

## Materials and Methods

Chloroplasts were isolated from spinach as previously described [12]; they were at least 75% intact and gave light- and $HCO_3^-$-saturated photosynthesis rates of 100—200 $\mu$mol $O_2$/mg chlorophyll per h. Measurements were performed at 19—20°C with chloroplasts suspended in 0.35 M sorbitol, 50 mM Tricine, and 0.3 mM $K_2HPO_4$ adjusted to pH 8.1. Samples contained 1600 units of catalase per ml except during $O_2$ uptake measurements when the catalase was replaced by 1.0 mM KCN. Methyl viologen, $K_3Fe(CN)_6$, $NaHCO_3$ and 3-phosphoglycerate additions were 25 $\mu$M, 1 mM, 10 mM, and 2 mM respectively. For ferricyanide reduction, thylakoids were prepared by osmotic rupture of intact chloroplasts as described in Ref. 11. Oxygen was measured with a Clark electrode (Yellow Springs Instruments model 5331) inserted into a stirred sample

suspension in a thermally jacketed cuvette. Absorbance (1 cm light path) was monitored with a single beam spectrophotometer; rapid changes were measured with a transient recorder (Data Laboratories model DL 922) and averaged by a PDP 11/34A computer. Signal averaging was also performed with a Fabritek 1052 averager. Actinic flashes were provided by illuminating the sample cuvette from opposing directions, at right angles to the measuring beam, with two EG&G FX201 xenon lamps (1.2 kV, 2 $\mu$F discharges). Red flashes, obtained with Schott RG 665 filters, had a 4 $\mu$s pulsewidth at half peak height and gave mostly single turnovers. $O_2$ uptake with methyl viologen or oxygen evolution with ferricyanide, and the 518 nm absorbance change were linear functions of chlorophyll concentration within the range used, and hence were flash-saturated.

## Results

*Flash-induced electron transport: Relation to flash frequency*

Fig. 1a displays the dependence of electron flow on flash frequency with either ferricyanide, 3-phosphoglycerate or $CO_2$ as the terminal electron acceptor. With ferricyanide, the $O_2$ evolution rate is a linear function of flash frequency and calculation of the flash yields in Fig. 1b gives a relatively constant value of 1.55 nequiv./mg chlorophyll or 1 $e^-$/650 chlorophylls. This is slightly larger than the photosynthetic unit size of 1 cytochrome *f* and 1 *P*-700 per 500 chlorophylls determined for spinach chloroplasts by redox titration [13,14]. However, the unit size is in good agreement with that determined for *Chlorella* [15] from flash yield data. Such an equivalence of unit size and flash yield indi-

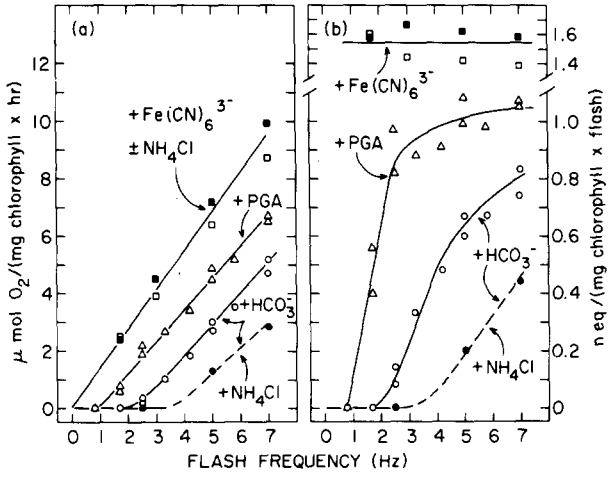


**Fig. 1. Flash frequency dependences of electron transport rates and flash yields for chloroplasts in the presence of various electron acceptors. The $O_2$ evolution rate (left) was measured with either ferricyanide (in the presence (■) and absence (□) of 3 mM $NH_4Cl$), or 3-phosphoglycerate (△) or $HCO_3^-$ (○) present as acceptor. Dashed plots (●) were obtained with $HCO_3^-$ and 1 mM $NH_4Cl$ present. The corresponding flash yields (right) were obtained by dividing these rates by the number of flashes per hour. Reaction conditions were as described in Materials and Methods. Samples containing $HCO_3^-$ were exposed to 3.5 min of continuous blue light (200 W/m$^2$) and a 1-min dark period before measurement of the rates induced by repetitive flashes.**

cates flash saturation and complete relaxation of the electron transport system between flashes. Additional evidence for relaxation of the electron carriers between flashes is provided by the inability of $NH_4Cl$ to accelerate coupled ferricyanide or methyl viologen reduction (data not shown) at flash frequencies below 7 Hz, in contrast to what is seen at higher flash frequencies and in saturating continuous light where a larger pH gradient limits the rate of electron flow. When methyl viologen was the acceptor, the flash yield was 1.2 nequiv./mg chlorophyll (data not shown).

A relatively constant flash yield for 3-phosphoglycerate reduction at frequencies above 2.5 Hz agrees with observations on proton gradient formation [16]. A significant pH gradient and phosphorylation rate must exist at low flash frequencies, since 3-phosphoglycerate or $HCO_3^-$ support $O_2$ evolution. Production of ATP is necessary for the conversion of 3-phosphoglycerate to *sn*-glycerol 1,3-bisphosphate, an electron acceptor which reoxidizes NADPH [17] thus permitting electron flow from $H_2O$ to $NADP^+$. Additional ATP is, of course, needed for conversion of ribulose 5-phosphate to ribulose 1,5-bisphosphate during $CO_2$ fixation. Comparison of the plots with 3-phosphoglycerate and $HCO_3^-$ in Fig. 1 shows that increased flash frequencies are needed to sustain continuous electron flow when the ATP/NADPH requirements for reduction of the acceptor are raised from 1.0 for 3-phosphoglycerate to 1.5 for $CO_2$. A higher rate of coupled proton translocation is apparently needed during $CO_2$ fixation to compensate for the increased utilization of ATP. The view that ATP supply is the limiting factor is also indicated by the need to use higher flash frequencies to sustain a given rate of $CO_2$ reduction when a partially uncoupling level of $NH_4Cl$ (1 mM) is present (dashed line). Similar results (not shown) were obtained with 3-phosphoglycerate as acceptor.

*Cytochrome turnover during 3-phosphoglycerate reduction*

Fig. 2 shows spectra of absorbance changes 100 $\mu$s and 1.6 ms after a flash, in chloroplasts reducing 3-phosphoglycerate (cf. Fig. 1). The electrochromic effect, characterized by a trough at 480 nm and a peak at 518 nm, is similar to that previously seen in intact chloroplasts under conditions which support cyclic electron flow [4]. Cytochrome *f* oxidation is indicated in the 100 $\mu$s curve by a bleaching at 554 nm. After 1.6 ms, absorbance differences around 554 nm and 564 nm are superimposed on the tail of the collapsing electrochromic effect.

A more detailed analysis of the cytochrome $\alpha$-band region appears in Fig. 3. Absorbance changes at 564 nm and 554 nm are presented in part (a) for times between 100 $\mu$s and 1 ms after the flash. From the spectra displayed in part (b), it is evident the changes in this time span correspond mainly to the oxidation of cytochrome *f* ($\lambda_{max}$ = 554 nm) and the reduction of cytochrome *b*-563 ($\lambda_{max}$ = 563.5 nm). The small rapid absorbance increase at 564 nm that occurs between 100 and 300 $\mu$s is kinetically similar to the reported oxidations of cytochrome *f* [18,19] and plastocyanin [19,20] and may be ascribed to the fractional absorbance increase from these components as they become oxidized (see Refs. 21, 22 and Table I). Likewise, the small apparent shift at 550 nm (Fig. 3) suggests some turnover of *C*-550. Similar spectra, showing the reoxidation of cytochrome *b*-563 and rereduction of cytochrome *f* were obtained

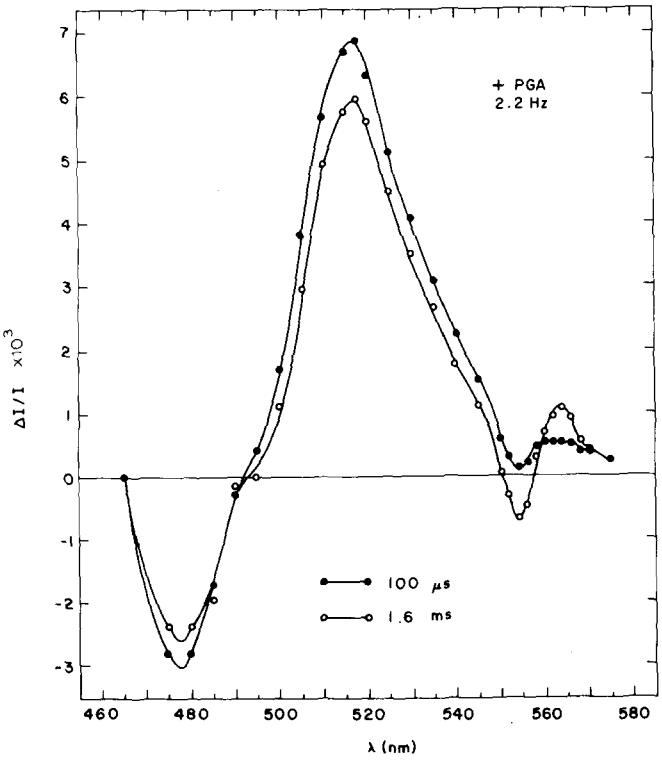


Fig. 2. Wavelength dependence of flash-induced absorbance changes 100 μs and 1.6 ms after the flash. Samples contained 3-phosphoglycerate and 52 μg chlorophyll/ml; otherwise conditions were as in Materials and Methods. The data at 100 μs (●) and 1.6 ms (○) for each wavelength represent the accumulated averages of between 128 and 1024 flashes given at 2.2 Hz.

when time intervals between 1 and 50 ms were analyzed in the above manner.

Estimation of the $t_{1/2}$ for cytochrome *f* oxidation gives a value of 130 μs, which is close to values reported for chloroplasts [18], leaves [23] and algae [19]. The $t_{1/2}$ of 600 μs for cytochrome *b*-563 reduction is lower than the value of 1.3 ms measured in broken chloroplasts [18]. Clearly, significant turnover of both cytochromes occurs during flash-driven electron flow to $NADP^+$ and subsequently 3-phosphoglycerate.

*Cytochrome turnover during $CO_2$ fixation*

Determination of flash yields for the cytochromes necessitates correction for absorbance changes from other pigments having overlapping absorbance in the α-band region. The differential extinction coefficients of these components and their estimated absorbance changes during $CO_2$ fixation in 7 Hz flashes are presented in Table I for the wavelengths 554, 564 and 570 nm. Calculations assumed a turnover of 0.75 nequiv./mg chlorophyll per flash, as indicated by the $O_2$ flash yield data in Fig. 1b. During repetitive flash excitation no turnover of cytochrome *b*-559 was observed (Fig. 3) and the predicted *C*-550 change (Table I, in brackets) was also small owing to the low extinction coefficient at 554 nm [24]; consequently, the electrochromic effect [4,18] and the oxida-

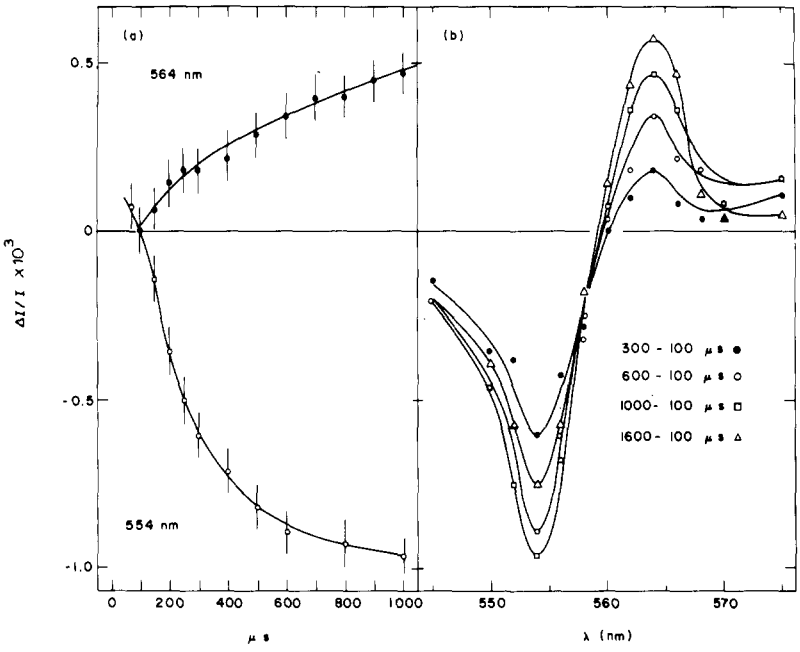


Fig. 3. Spectra of the absorbance changes occurring between 100 μs and 1.6 ms after the flash. Conditions as in Fig. 2. In part (a) the relative absorbance change between 100 μs and 1.0 ms is given for the wavelengths 564 (●) and 554 nm (○). Absorbance spectra, in part (b), are plotted for time intervals corresponding to 100 to 300 μs (●), 100 to 600 μs (○), 100 to 1000 μs (□) and 100 to 1600 μs (△).

tions of *P*-700 [25] and plastocyanin [22] must account for most of the non-cytochrome absorbance changes at these wavelengths. From the 1 : 1 stoichiometry between $P\text{-}700^+$ reduction and plastocyanin oxidation [20], and the kinetics (70—80% reduction of $P\text{-}700^+$ after 1 to 2 ms [20]) can be computed their absorbance contributions for the 1 to 2 ms interval after the flash (Table I). The electrochromic effect can be estimated from responses measured

TABLE I

EXTINCTION COEFFICIENTS AND ESTIMATED ABSORBANCE CHANGES

Extinction coefficients ($\epsilon$ = $mM^{-1} \cdot cm^{-1}$) were derived from the spectra referenced. Absorbance contributions ($(\Delta I/I) \times 10^5$) were estimated as described in the text, for $CO_2$ fixation in 7 Hz flashes (51 μg chlorophyll/ml) and appear in brackets.

| Component | | Wavelength | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | 554 nm | | 564 nm | | 570 nm | | |
| | | $\epsilon$ | $A$ | $\epsilon$ | $A$ | $\epsilon$ | $A$ | |
| Cytochrome *b*-559 | red-ox | 6.6 | [0] | 6.6 | [0] | —1.0 | [0] | 39,40,43 |
| *C*-550 | red-ox | —1.7 | [—15] | 0.0 | [0] | 0.0 | [0] | 24 |
| *P*-700 | ox-red | 3.2 | [9] | 2.6 | [7] | 1.8 | [4] | 25 |
| Plastocyanin | ox-red | 2.9 | [18] | 3.4 | [21] | 3.8 | [24] | 19,20,22 |
| *P*-518 | $\%\Delta A_{518nm}$ | 6.8% | [26] | 5.3% | [20] | 4.6% | [17] | 4,18 |
| Cytochrome *b*-563 | red-ox | 0.5 | — | 19.0 | — | 5.0 | — | 39,41—43 |
| Cytochrome *f* | ox-red | —17.7 | — | 2.0 | — | 2.5 | — | 21,39,43 |

at 518 nm [4,18], such as those shown in Fig. 4; calculated absorbance contributions from *P*-518 are shown in Table I for the steady state phase of $CO_2$-fixation (see Fig. 4b). The sums of the absorbance changes of *P*-700$^+$/*P*-700, oxidized/reduced plastocyanin, and *P*-518 are nearly the same at all three wavelengths; thus, absorbance changes at 554 nm and 564 nm may be corrected for contributions from these pigments by subtraction of the absorbance change at 570 nm.

Flash-induced cytochrome turnovers and the $O_2$ evolution curve for chloroplasts preilluminated with added $HCO_3^-$ are presented in Fig. 5. Fig. 5a illustrates the absorbance transients observed at 564 nm and 554 nm and, in the bottom trace, $O_2$ evolution measured under identical conditions. In Fig. 5b, traces are shown of cytochrome *b*-563 and cytochrome *f* turnovers after subtraction of the change at 570 nm. For comparison, traces are also shown in part (c) for chloroplasts in the presence of 1 mM $NH_4Cl$, which depressed the rate of $O_2$ evolution (cf. Fig. 1). This effect can be attributed to a partial uncoupling of electron flow from phosphorylation, as evidenced by the more rapid rates of cytochrome *f* reduction and cytochrome *b*-563 oxidation.

Calculations of the flash yields from Fig. 5b, using the differential extinction coefficients relative to 570 nm for cytochromes *b*-563 and *f* gave values of 0.30 and 0.79 nequiv./mg chlorophyll, respectively. Alternatively, the observed changes at 554 and 564 nm in Fig. 51 could be corrected by subtraction of the absorbances listed in Table I. This procedure gives flash yields of 0.24 and 0.82 for cytochromes *b*-563 and *f*, respectively. Such agreement between the results of the two correction procedures suggests that either can be employed with reasonable accuracy.

The significant cytochrome *b*-563 turnover and the inability to account for cytochrome *f* turnover by Photosystem II activity alone provide strong evidence for cyclic electron flow during $CO_2$ fixation. From the flash yield and the relaxation time given in Fig. 5b, a maximum turnover rate through cytochrome *f* of about 400 μequiv./mg chlorophyll per h can be estimated, which corresponds well with $O_2$ evolution rates of 100 μmol/mg chlorophyll per h observed in continuous light. A similar estimate of cytochrome *b*-563 turnover gives a value of 96 μequiv./mg chlorophyll per h and corresponds to a phosphorylation rate of 32 μmol ATP/mg chlorophyll per h if an efficiency of

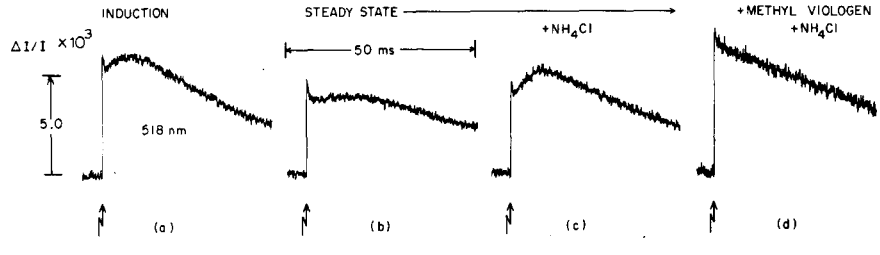


**Fig. 4. Kinetic traces for the flash induced absorbance change at 518 nm during and following induction of photosynthesis. Traces were the accumulated averages of 128 flashes. Samples contained 51 μg chlorophyll/ml and 10 mM $NaHCO_3$ in 'reaction buffer'. Other conditions were: (a), dark adapted then flashed at 2.5 Hz; (b), preilluminated then flashed at 7 Hz; (c), as (b) but with 1 mM $NH_4Cl$ present. In trace (d) $HCO_3^-$ was replaced by 25 μM methyl viologen and 3 mM $NH_4Cl$; the flash frequency was 7 Hz.**

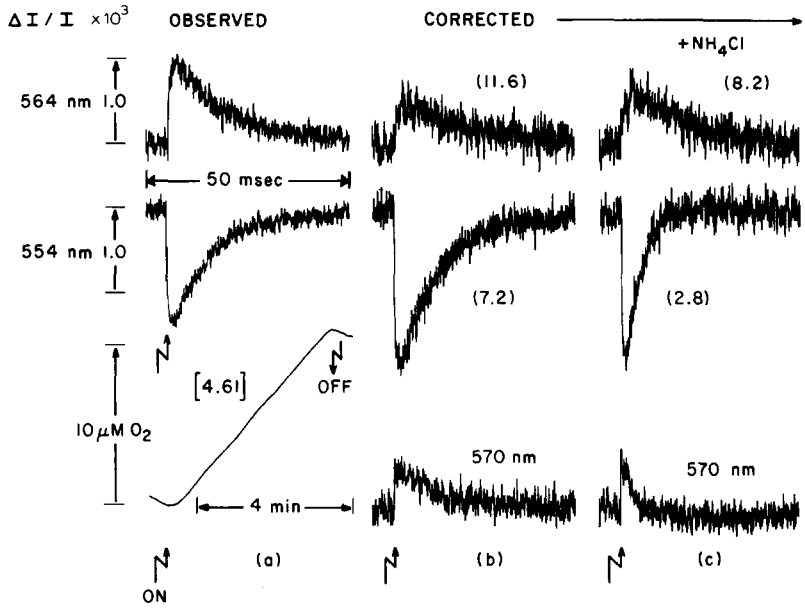


Fig. 5. Flash-induced cytochrome turnover and $O_2$ evolution in chloroplasts during $CO_2$-fixation. Samples containing 10 mM $HCO_3^-$ were illuminated before repetitive flashes were given at 7.0 Hz as in Fig. 1. Chlorophyll concentrations were 40 μg/ml and 51 μg/ml for $O_2$ and absorbance measurements, respectively. Absorbance traces in part (a) represent the accumulated averages of 512 flashes; $O_2$ evolution was recorded during a 4-min period of repetitive flashes. In parts (b) and (c) the cytochrome responses were obtained by subtracting the 570 nm absorbance averaged over 512 sweeps from that measured after 512 sweeps at 564 or 554 nm. The 570 nm absorbance change is shown in the bottom traces. Arrows indicate the onset of a flash in the case of absorbance changes and the onset or termination of flashes for $O_2$ evolution. Numbers in parentheses give the computed first order half-times, in ms, for cytochrome *b*-563 oxidation and cytochrome *f* reduction. The rate of $O_2$ evolution in μmol/mg chlorophyll per h is given in square brackets. $NH_4Cl$ (1 mM) was present in part (c).

1 $H^+/e^-$ or 0.66 ATP/2$e^-$ [26] is assumed for the coupling site located between plastoquinone and cytochrome *f* [3]. Such a rate could provide the ATP needed to supplement that arising from coupled (ATP/2$e^-$ = 1.33) linear electron flow [27] in chloroplasts fixing $CO_2$. If the recently discovered additional slow charge separation across the thylakoid [4,28,29] results in a total translocation of 2 $H^+/e^-$ in the cyclic pathway, the true phosphorylation rate would be twice that estimated above.

*Determination of relative electron transport activities from flash yields*

Estimations of the relative electron transport activities can be made from Table II where the flash yields for cytochrome turnovers are compared with measurements of Photosystem II activity under various conditions. With 3-phosphoglycerate, the sum of the flash yields for cytochrome *b*-563 and Photosystem II more than adequately accounts for cytochrome *f* turnover. This is consistent with earlier studies [30] showing that 3-phosphoglycerate reduction is not accompanied by pseudocyclic electron flow. As a check on the ability of these preparations to support pseudocyclic flow, $O_2$ uptake was measured in the presence of uncoupler and KCN. The pseudocyclic flash yield of 0.22 nequiv./mg chlorophyll (Table II) contributes almost half of the overall

TABLE II

FLASH YIELD FOR PHOTOSYSTEM II, CYCLIC, PHOTOSYSTEM I AND PSEUDOCYCLIC ACTIVITIES

Flash yields (±0.05) are given in nequiv./mg chlorophyll for $O_2$ evolution, cytochrome *b*-563 reduction, cytochrome *f* oxidation, $O_2$ uptake and the extent of the slow rise in *P*-518, observed in repetitive flashes. Bracketed values: corrected using response at 570 nm. *P*-518 units: $(\Delta I/I) \times 10^3$.

| Conditions | Activity: Flash yield: | PS II $O_2$ evolution | Cyclic Cyt *b*-563 | PS I Cyt *f* | Pseudo cyclic $O_2$ uptake | Cyclic Slow *P*-518 |
|---|---|---|---|---|---|---|
| +3-phosphoglycerate, 2.5 Hz | | 0.90 | 0.20 | 0.96 | — | 0.9 |
| +KCN, +$NH_4Cl$ (3 mM), 7 Hz | | — | 0.25 | 0.51 | 0.22 | 0.9 |
| +$HCO_3^-$, 2.5 Hz (induction) | | 0.25 | 0.40 | 0.74 | — | 1.2 |
| +$HCO_3^-$, 7 Hz (steady state) | | 0.75 | 0.24 [0.30] | 0.82 [0.79] | — | 0.8 |
| +$HCO_3^-$, +$NH_4Cl$ (1 mM) | | 0.44 | 0.39 [0.45] | 0.78 [0.76] | — | 1.7 |

flow through cytochrome *f*, which is otherwise attributable to cycled electrons. In all other cases presented in Table II, the sum of the flash yields suggests that pseudocyclic flow does not contribute significantly to cytochrome *f* turnover. Pseudocyclic flow is not directly measurable in these cases owing to the presence of catalase.

There is a large demand for ATP during the induction phase of photosynthesis [31] to supply the Calvin cycle with phosphorylated intermediates, and high rates of cyclic turnover might be predicted. When dark adapted chloroplasts were exposed to low frequency flashes (2.5 Hz) in the presence of $HCO_3^-$, a low flash yield for oxygen evolution of 0.25 nequiv./mg chlorophyll was recorded and this remained constant for up to 15 min. The flash yield for cytochrome *b*-563 was high as predicted, at 0.40 nequiv/mg chlorophyll. Pseudocyclic flow was not directly measurable during photosynthetic induction; however, the flash yield for pseudocyclic flow must be low as shown by the additivity of linear and cyclic turnovers to give almost the entire cytochrome *f* turnover (Table II). An additional feature of the induction phase is a pronounced slow absorbance rise during relaxation of the 518 nm transient (cf. Fig. 4a) which has previously been correlated with rapid cyclic turnover in intact chloroplasts [4,28]. To estimate the amplitude of this change, the decay rate of the 518 nm absorbance was recorded under conditions strongly favouring linear electron flow (with methyl viologen and 3 mM $NH_4Cl$ added). The result, shown in Fig. 4d, was normalized at 1 ms after the flash and subtracted from Fig. 4a, b, and c, to give the amplitudes (Table II). Following elimination of the induction phase by preillumination, the cytochrome *b*-563 flash yield was a smaller but significant fraction of that recorded for cytochrome *f*, and the slow rise in the electrochromic effect was decreased by 33%. Subsequent addition of $NH_4Cl$ increased the rates of both cytochrome *b*-563 oxidation and cytochrome *f* reduction as seen in Fig. 5; moreover, the extent of cytochrome *b*-563 reduction and the slow rise at 518 nm were also increased. Similar effects were noted under conditions supporting only cyclic flow [4,28]. The above data show that cyclic electron transfer involving cytochromes *b*-563 and *f* is increased when the demand for ATP relative to NADPH rises, or when the coupling efficiency is lowered.

## Conclusions

The large cytochrome *f* turnover observed here is consistent with cytochrome *f* participating in both linear and cyclic electron transfer chains. Estimation of the cytochrome *f* turnover in Fig. 5 gives a value (400 μequiv./mg chlorophyll/h) sufficient for electron flow in chloroplasts fixing $CO_2$ at a substantial rate. With uncoupler (Table II and Fig. 5c) a turnover of 1000 μequiv./mg chlorophyll per h is indicated, which is comparable to maximum rates of linear electron flow in uncoupled chloroplasts [32]. These are minimal estimates since the observed cytochrome *f* yield per flash is only 43% of the maximum determined by redox titration [13,14]. Measurements elsewhere of a low flash yield of cytochrome *f* suggested its functioning in parallel with plastocyanin [20]. However, more recent data show that the observed low yield per flash may be due to a combination of an equilibration with plastocyanin [19] and a rapid reduction by the Rieske iron-sulfur center [33]. When such effects are taken into consideration, the flash yields reported in the present work are close to the theoretical limit.

The cyclic contribution to photosynthesis is evident from the flash yield stoichiometries of 0.75 (from Photosystem II): 0.24 cytochrome *b*-563: 0.82 cytochrome *f*. At a flash frequency of 7 Hz (approximating the low light levels used in quantum yield measurements), this would correspond to the quantum requirements of 11—12 determined for $O_2$ evolution or $CO_2$ fixation [30,34].

Uptake of $O_2$ was also measured in this study but the flash yield was considerably less than the yields for linear and cyclic electron transfers through cytochrome *f*. Early mass spectroscopic observations of concurrent $O_2$ evolution and uptake suggested that pseudocyclic electron flow might be an important source of ATP [30]; however, recent measurements [35,46] have shown that pseudocyclic activity is quite low during steady state photosynthesis in chloroplasts. Furthermore, much of the $O_2$ uptake has been attributed to glycollate formation by the oxygenase activity of ribulose 1,5-bisphosphate carboxylase [36].

Electron flow in thylakoids is well known to be regulated by the magnitude of the transmembrane proton gradient, which affects the rate of reoxidation of plastoquinone and hence the rate of rereduction of cytochrome *f* [2,3]. The accelerations in the rates of cytochrome *b*-563 oxidation and cytochrome *f* reduction, which occur with uncoupler or low ATP/ADP ratios, indicate that such a control point exists in intact chloroplasts and is demonstrable during assimilation of $CO_2$.

The relative activities of linear and cyclic electron flow appear to be determined by the redox state of a common (plastoquinone?) pool. Table II and Fig. 5 show that increased cyclic turnover accompanies the decline in Photosystem II activity that results from poor regeneration of $NADP^+$, with consequent closure of Photosystem II traps. High imposed NADPH/$NADP^+$ ratios can also partially close Photosystem II traps via reduction of the plastoquinone pool through part of the cycle involving ferredoxin and cytochrome *b*-563 [11]. Photosystem I will remain active under these conditions as the midpoint potential of its acceptor [37] is lower than that of the Photosystem II acceptor complex [38]. One would thus expect cyclic electron flow to persist or even be

enhanced as Photosystem II is progressively shut off. Such a competitive interaction between cyclic and linear pathways might be responsible for maintainance of an ATP/NADPH production stoichiometry appropriate to the needs of the chloroplast.

## Acknowledgement

This research was carried out at Brookhaven National Laboratory under the auspices of the United States Department of Energy.

## References

1 Arnon, D.I. and Chain, R.K. (1977) in Photosynthetic Organelles, Special Issue, Plant Cell Physiol., 129—147
2 Böhme, H. and Cramer, W.A. (1972) Biochim. Biophys. Acta 283, 302—315
3 Hauska, G., Reimer, S. and Trebst, A. (1974) Biochim. Biophys. Acta 357, 1—13
4 Slovacek, R.E., Crowther, D. and Hind, G. (1979) Biochim. Biophys. Acta 547, 138—148
5 Schurmann, P., Buchanan, B.B. and Arnon, D.I. (1971) Biochim. Biophys. Acta 267, 111—124
6 Huber, S.C. and Edwards, G.E. (1977) FEBS Lett. 79, 207—211
7 Slovacek, R.E., Mills, J.D. and Hind, G. (1978) FEBS Lett. 87, 73—76
8 Tanner, W., Loos, E. and Kandler, O. (1966) in Currents in Photosynthesis (Thomas, J.B. and Goedheer, J.C., eds.), pp. 243—250, Donker, Rotterdam
9 Kaiser, W. and Urbach, W. (1976) Biochim. Biophys. Acta 423, 91—102
10 Heber, U. (1969) Biochim. Biophys. Acta 180, 302—319
11 Mills, J.D., Crowther, D., Slovacek, R.E., Hind, G. and McCarty, R.E. (1979) Biochim. Biophys. Acta 547, 127—137
12 Slovacek, R.E. and Hind, G. (1977) Plant Physiol. 60, 538—542
13 Heber, U., Boardman, N.K. and Anderson, J.M. (1976) Biochim. Biophys. Acta 423, 275—292
14 Boardman, N.K. (1971) Methods Enzymol. 23, 268—276
15 Emerson, R. and Arnold, W. (1932) J. Gen. Physiol. 16, 191—205
16 Inoue, Y., Kobayashi, Y., Shibata, K. and Heber, U. (1978) Biochim. Biophys. Acta 504, 142—152
17 Bassham, J.A. (1964) Annu. Rev. Plant Physiol. 15, 101—120
18 Dolan, E. and Hind, G. (1974) Biochim. Biophys. Acta 357, 380—385
19 Bouges-Bocquet, B. (1977) Biochim. Biophys. Acta 462, 362—370
20 Haehnel, W. (1977) Biochim. Biophys. Acta 459, 418—441
21 Bendall, D.S., Davenport, H.E. and Hill, R. (1971) Methods Enzymol. 23, 327—344
22 Katoh, S., Shiratori, I. and Takamiya, A. (1962) J. Biochem. 51, 32—40
23 Hildreth, W.W. (1968) Biochim. Biophys. Acta 153, 197—202
24 Melis, A. and Schreiber, U. (1979) Biochim. Biophys. Acta 547, 47—57
25 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160—171
26 Izawa, S. and Pan, R.L. (1978) Biochem. Biophys. Res. Commun. 83, 1171—1177
27 Junge, W. (1977) Annu. Rev. Plant Physiol. 28, 503—536
28 Crowther, D., Mills, J.D. and Hind, G. (1979) FEBS Lett. 98, 386—390
29 Mitchell, P. (1976) J. Theor. Biol. 62, 327—367
30 Egneus, H., Heber, U., Matthiesen, U. and Kirk, M. (1975) Biochim. Biophys. Acta 408, 252—268
31 Walker, D.A. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 235—278, Elsevier North-Holland Biomedical Press, Amsterdam
32 Lilley, R.McC., Fitzgerald, M.P., Rienits, K.G. and Walker, D.A. (1975) New Phytol. 75, 1—10
33 Koike, H., Satoh, K. and Katoh, S. (1978) Plant Cell Physiol. 19, 1371—1380
34 Heber, U. (1976) J. Bioenerg. Biomemb. 8, 157—172
35 Marsho, T.V., Behrens, D.W. and Radmer, R.J. (1979) Plant Physiol. 64, 656—659
36 Berry, J.A., Osmond, C.B. and Lorimer, H.G. (1978) Plant Physiol. 62, 954—967
37 Ke, B., Hansen, R.E. and Beinert, H. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2941—2945
38 Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188—201
39 Cramer, W.A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133—172
40 Lach, H.J. and Böger, P. (1977) Z. Naturforsch. 32, 75—77
41 Stuart, A. and Wasserman, A. (1973) Biochim. Biophys. Acta 314, 284—297
42 Nelson, N. and Neumann, J. (1972) J. Biol. Chem. 247, 1817—1824
43 Hind, G. and Nakatani, H.Y. (1970) Biochim. Biophys. Acta 216, 223—225
44 Arnon, D.I. and Chain, R.K. (1977) FEBS Lett. 82, 297—302
45 Arnon, D.I. and Chain, R.K. (1979) FEBS Lett. 102, 133—138
46 Heber, U., Egneus, H., Hanck, U., Jensen, M. and Köster, S. (1978) Planta 143, 41—49