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INTACT CHLOROPLAST ELECTRON FLOW

EFFECTS OF RIBOSE 5-PHOSPHATE

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Additions of ribose 5-phosphate to intact spinach chloroplasts were used to probe the effects of ADP regeneration on pH-gradient formation and electron-transfer reactions. In weakly illuminated chloroplasts, the ATP/ADP ratio dropped by 64% and the transthylakoid pH gradient decreased by a minimum of 0.2 units in response to ribose 5-phosphate. Nitrite reduction increased 2-fold while, under conditions of cyclic electron flow, the half-time for cytochrome *f* reduction decreased by a factor of two from 4.1 to 1.9 ms. The results suggest that metabolic ATP consumption, during the conversion of ribulose 5-phosphate to ribulose 1,5-bisphosphate, enhances electron transfer between plastohydroquinone and cytochrome *f* through decreases in the transthylakoid pH gradient caused by phosphorylation of ADP.

Electron transport in chloroplast thylakoids is known to be regulated by the buildup of a transmembrane H^+ gradient [1] which affects the rate of plastohydroquinone oxidation [2] and hence the rate of cytochrome *f* reduction [3]. This phenomenon can also be demonstrated by accelerations in either ferricyanide or methyl viologen reduction [4,5] seen when uncouplers are used to collapse the pH gradient. Likewise, electron flow is increased when the H^+ gradient is dissipated during phosphorylation of ADP [5,6] by the chloroplast coupling factor CF_1 [7]. With intact chloroplasts, regeneration of the ADP pool normally occurs as a result of ATP utilization in the phosphorylation of 3-phosphoglycerate and ribulose 5-phosphate substrates during CO_2 fixation [8]. However, direct demonstrations that ATP utilization affects electron-transfer rates in vivo [9] are difficult, since most assays involve substrates (e.g., CO_2, 3-phosphoglycerate or oxaloacetate) which are dependent upon an integration of enzyme functions for the continuous regeneration of $NADP^+$. Enzymes such as fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, glyceraldehyde-3-phosphate dehydrogenase, phosphoribulokinase and malate dehydrogenase are light activated through a reductive mechanism [10] and may also be influenced by stromal Mg^{2+} [11] and H^+ [12] levels. Consequently, rate limitations in electron transfer are not easily distinguishable from metabolic processes which regenerate the cofactors ADP and $NADP^+$.

This study describes one approach to the problem. Net turnover of the Calvin cycle was inhibited by the absence of added CO_2 to chloroplast suspensions and measurements of electron flow were restricted to assays of nitrite reduction [9] or cytochrome *f* turnover in the cyclic pathway [3] as production of $NADP^+$ was blocked. The conversion of ATP into ADP was initiated by

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Tricine, *N*-tris(hydroxymethyl)-methylglycine; Chl, chlorophyll.

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additions of ribose 5-phosphate to promote the reaction sequence:

Ribose 5-phosphate→

ribulose 5-phosphate→ribulose 1,5-bisphosphate

$$\overset{\text{ATP}}{\rightarrow}\ \overset{\text{ADP}}{}$$

This was previously shown [13] to be an effective ATP sink by the inhibition of 3-phosphoglycerate reduction caused by a diminished ATP/ADP ratio when ribose 5-phosphate was added to chloroplasts.

Chloroplasts were isolated from spinach as described earlier [14]. Intactness, which ranged from 80 to 90%, was assayed by the ratio of uncoupled ferricyanide reduction before and after osmotic shock. 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 catalase/ml and 20–28 μg Chl/ml or in the case of absorbance measurements 50 μg Chl/ml. Oxygen evolution was assayed polarigraphically with 2.0 mM KNO_2 as the electron acceptor. Values for ΔpH were calculated according to Schuldiner et al. [15] from the quenching of 9-aminoacridine fluorescence corrected for a binding term [16]. Adenine nucleotides were determined by the luciferin-luciferase method following $HClO_4$ extraction [17]. Flash-induced absorbance changes due to cytochrome *f* ($\lambda_{max} = 554$ nm) and P-518 (an electrochromic band shift of pigment absorbance at 518 nm) were measured with a single-beam spectrophotometer coupled to a Data Lab DL 922 transient recorder. A Digital Equipment Corp. PDP 11/34A computor was used for signal averaging as described in Ref. 18.

The results of Table I(A) agree with observations that coupled nitrite reduction is accelerated by NH_4Cl or CCCP [9] at less than saturating light intensities as it does not involve the utilization of ATP. Rate increases are also seen when antimycin, an inhibitor of cyclic electron flow [17], is used in place of gramicidin or NH_4Cl. A similar rate enhancement would be expected if the uncoupled phenomena could be duplicated with an endogenous substrate phosphorylation system for rapidly regenerating ADP. Evidence for this is

TABLE I

EFFECTS OF UNCOUPLERS AND RIBOSE 5-PHOSPHATE ON NITRITE REDUCTION

Samples containing 28 μg Chl/ml and 1600 units catalase/ml were illuminated with either low (20 W/m^2) or high (200 W/m^2) intensities of red (Corning CS-2-58) light. Oxygen-evolution rates were recorded 4 min after the onset of illumination. Additions to chloroplasts incubated either in the presence or absence of 2.0 mM KNO_2 were as indicated.

Conditions	Oxygen evolution rates (μmol O_2/mg Chl per h)	
	Low light	High light
(A) Chloroplasts + KNO_2 (2.0 mM)	4	5
+ NH_4Cl (5.0 mM)	14	27
+ Gramicidin D (12 μM)	12	25
+ Antimycin A (5.0 μM)	11	17
+ Ribose 5-phosphate (2.0 mM)	13	22
(B) Chloroplast control	0	0
+ Ribose 5-phosphate (2.0 mM)	0	0

given in Table I(A) where an addition of ribose 5-phosphate is seen to triple the O_2-evolution rate in a manner analogous to that of uncouplers. Phosphoribulokinase activity in dark-adapted chloroplasts reportedly [19,20] varies between 60 and 700 μmol/mg Chl per h with further increases caused by 1 min of weak illumination [20]. Accordingly, all measurements were performed after a 4 min light exposure to ensure elevated enzyme activity and establishment of a rate-limiting pH gradient. Although nitrite has been suggested [26] as a possible inhibitor of reducibly activated enzymes [10], there is no indication in the table for inactivated enzymes preventing ribose 5-phosphate from having an effect. Evidently, basal activity of phosphoribulokinase (see above) is more than adequate for the rates of electron flow observed.

In Table II, values for chloroplast adenine nucleotide levels are compared with the transthylakoid pH gradient (ΔpH) estimated for samples illuminated as in Table I(B) where nitrite was omitted. Cyclic electron flow would be prevalent under such conditions [17]. Both the ATP/ADP ratio and ΔpH show a decline upon addition of

TABLE II

THE EFFECTS OF RIBOSE 5-PHOSPHATE ON ADENINE NUCLEOTIDE LEVELS AND ΔpH UNDER WEAK ILLUMINATION

Conditions were as in Table IB except that 10 μM 9-aminoacridine was added to the samples used for ΔpH determinations. Calculations of ΔpH (see Ref. 16) were based on 9-aminoacridine fluorescence traces similar to those found in Fig. 4 of Ref. 24. The ATP, ADP and AMP contents of dark-adapted chloroplasts were 6.7, 20.4 and 10.1 nmol/mg Chl, respectively.

Conditions	ATP (nmol/mg Chl)	ADP (nmol/mg Chl)	ATP/ADP	ΔpH
Control	19.1	17.5	1.09	4.02
+2.0 mM ribose 5-phosphate	9.3	22.9	0.41	3.80

ribose 5-phosphate. Calculations of ΔpH were based on the assumption [16] that alkalinization of the stromal compartment raises the pH toward 8.1, in near equilibrium with the external medium at this incubation pH [12]. With the weak light used (e.g., 20 W/m^2), stromal pH changes may in fact be less alkaline than when moderate to strong illumination is used [12], thus causing a small overestimate of the actual ΔpH. However, the change in ΔpH can be regarded as a minimum, since only the relative decreases in thylakoid acidification was measured. A corresponding decline in stromal alkalinization would further diminish the lower ΔpH value caused by ribose 5-phosphate.

The electrochromic and cytochrome *f* absorbance changes are presented in Fig. 1 as monitors of cyclic electron-transport activity [3,18] in samples illuminated with a series of short red flashes. A train of 50 flashes was used to prereduce

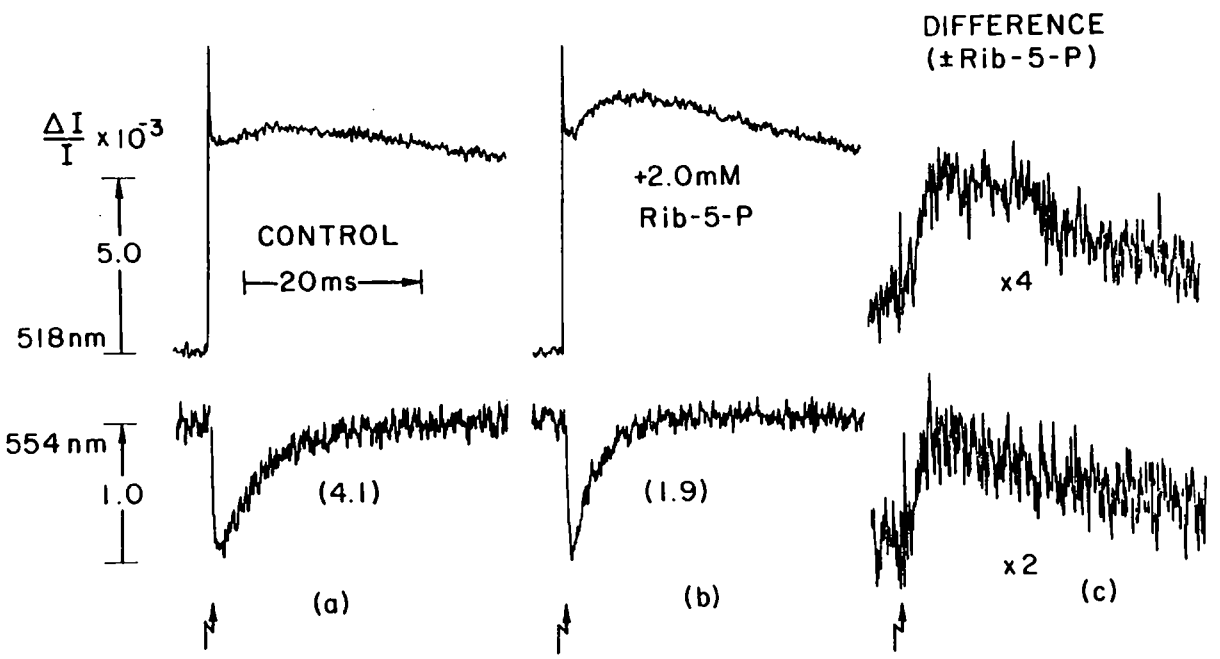

Fig. 1. Ribose 5-phosphate (Rib-5-P) effect on the flash-induced absorbance changes of P-518 and cytochrome *f*. Samples containing 50 μg Chl/ml in medium supplemented with 2.0 mM K_2HPO_4 were preilluminated with 50 flashes before measurements. Changes at 518 nm represent the average of 128 flashes while those at 554 nm are the accumulated average of 512 flashes after correction for the P-518 component as described in Ref. 3. Traces plotted on the expanded scales in c were obtained by subtraction of the traces in a from those in b. Flash frequency was 0.5 Hz.

the $NADP^+$ pool and further activate phosphoribulokinase before the actual sequence of flashes employed in measurements. In addition, the assay mixtures contained 2.0 mM K_2HPO_4 to facilitate export of triose phosphate from chloroplasts [21] via the phosphate translocator [22]. While this precaution insured a more complete inhibition of endogenous pentose phosphate production, it later proved unnecessary as similar results (not shown) were obtained without the high phosphate addition. Illustrated in Fig. 1a are traces for control chloroplasts which exhibit a slow rise superimposed on the decaying P-518 absorbance change. Cytochrome *f* reduction is moderately fast with a corresponding $t_{1/2}$ of 4.1 ms following a rapid oxidation caused by the flash. This may be attributed to use of a relatively low (0.5 Hz) flash frequency for limiting H^+ translocation and hence the buildup of a maximum pH gradient (see Ref. 3) for comparable results at 2.0 Hz). Traces in Fig. 1b are from the same experiment except that chloroplasts were incubated with 2.0 mM ribose 5-phosphate. A significantly faster reduction of cytochrome *f* is evidenced by the drop in $t_{1/2}$ to 1.9 ms, while P-518 shows a marked enhancement in the slow absorbance increase. Note these changes, displayed for clarity as difference traces on the expanded scales in Fig. 1c, are similar to earlier spectroscopic results [3,18] with uncouplers.

This study presents evidence for an interaction between the metabolic and electron-transport pathways which is apparently related to H^+ movements across the thylakoid membrane when regenerated ADP is phosphorylated. Nitrite was shown [9,23] to support linear electron flow coupled to H^+ uptake when added to intact chloroplasts. Since both high light and uncoupler (i.e., gramicidin, NH_4Cl or CCCP [9]) or antimycin are necessary to produce rapid reduction rates, it is unlikely that nitrite permeability is limiting electron flow. Rather, comparisons of the data in Table I and Fig. 1 with those found in Refs. 3, 9, 18 and 23 would indicate that a large pH gradient is responsible for the inhibition of linear and cyclic electron flows under conditions where ATP is not utilized. This is presumably due to a common coupling site, located between plastoquinone and cytochrome *f*, in both pathways [3]. Additions of ribose 5-phosphate, which should facilitate ADP regeneration, have the effect of accelerating electron flow similar to an uncoupler. Regardless of whether the decreased ATP/ADP ratio is due to a decline in the inorganic phosphate concentration with ribulose 1,5-bisphosphate buildup [25] or more directly indicates rapid ATP consumption [11], it reflects an increased esterification of phosphate through turnover of the ATP pool. The phosphorylation of ADP is known to diminish ΔpH in thylakoids [1] and its importance in promoting rapid electron-flow rates in vivo can be inferred from studies [4] showing that CO_2 assimilation in intact chloroplasts often approaches the uncoupled ferricyanide reduction rates of their osmotically lysed counterparts. The above method for regenerating ADP through an endogenous mechanism gives results generally consistent with a correlation between phosphorylation and electron flow, thus it may supplement studies which make use of uncouplers to investigate photophosphorylation in intact chloroplasts.

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