ATP DRIVEN REVERSE ELECTRON TRANSPORT IN CHLOROPLASTS

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1. Introduction

The light triggered thiol dependent ATPase of isolated chloroplasts [1] has been considered to operate through the reversal of the latter stages of ATP formation during photophosphorylation. This view has been supported by the observations that an ATP–P_i exchange reaction accompanied the appearance of light triggered ATPase and could be considered a partial reaction of the ATPase [2, 3]. More recently [4] it has been shown that in the dark the hydrolysis of ATP by the light triggered ATPase will cause an uptake by chloroplasts of protons from the medium, leading to the formation of a proton concentration gradient [5].

We wish to report that isolated lettuce chloroplasts, treated so as to show light triggered ATPase activity, exhibit an ATP dependent enhancement of chlorophyll fluorescence which appears to be the consequence of reversed electron flow in the chloroplast driven by ATP hydrolysis.

A site for ATP formation in chloroplasts has been located between photosystem II and cytochrome *f* [6, 7]. If reversed electrom flow driven by ATP occurs it would be expected to lead to some reduction of components of the electron transport chain close to Photosystem II. Reduction of Q, the hypothetical primary electron acceptor for Photosystem II, could be easily and sensitively followed as an increase of chlorophyll fluorescence yield [8, 9]. The oxidized state of Q is presumed to quench chlorophyll fluorescence while the reduced state does not. Our experiments show an increased chlorophyll fluorescence and therefore a reduction of Q on adding ATP to light triggered lettuce chloroplasts.

Abbreviations:
PMS = phenazine methosulfate;
DTT = dithiothreitol;
DCMU = 3-(3,4-dichlorophenyl)-1,1,dimethylurea;
NQNO = 2-n-nonyl-4-hydroxyquinoline-*N*-oxide;
BDHB = n-butyl-3,5-diiodo-4-hydroxybenzoate.

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2. Materials and methods

Once washed chloroplasts were prepared from lettuce (*Lactuca sativa* Var. romaine) as previously described [10]. For light triggering they were suspended in a 1 cm square cuvette at a final concn. at or near 40 μg chlorophyll per ml, in 1.8 ml of a standard reaction mixture containing: sucrose, 90 mM; NaCl, 45 mM; Tris-HCl, pH 7.8, 22.5 mM; DTT, 5 mM; $MgCl_2$, 5 mM; and PMS, 2.5 μM. The chloroplasts were then light triggered for 2 min by light from a Xenon lamp filtered through 2 cm saturated $CuSO_4$ solution, with an intensity of 8.3×10^5 ergs $\times$ sec^{-1} at the cuvette surface. Within 2–3 sec after turning off the triggering light, the shutter admitting the exciting light for the fluorescent measurements was opened and fluorescence measurements recorded on either an oscilloscope or a chart recorder. ATP (in 5–10 μl) was routinely added to the reaction mixture 30 sec after turning off the triggering light. Other substances were added either before, with or after ATP addition. The reaction mixture was stirred momentarily while making additions.

The exciting light for fluorescence measurements was from a tungsten lamp of a projector passed through a Corning C.S. 4-96 and a Baird Atomic interference filter peaking at 550 nm (half band width 20 nm,

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blocked to infinity). Intensity was controlled by varying the voltage applied to the lamp. The usual intensity at the reaction cuvette surface was about 600 ergs × cm^{-2} × sec^{-1}. Emitted fluorescent light was detected with a photomultiplier (EM1, S20 sensitivity) after passing through a Schott RG 665 and Corning CS 4-77 filters.

Chlorophyll was measured according to Arnon [11].

3. Results and discussion

Fig. 1 demonstrates some of the characteristics of the light triggered ATP dependent fluorescence changes. Immediately after light triggering the level of fluorescence was high but falling so that by about 30 sec a steady state level was approached. ATP (1 mM) produced a marked rise in fluorescence yield (fig. 1A)

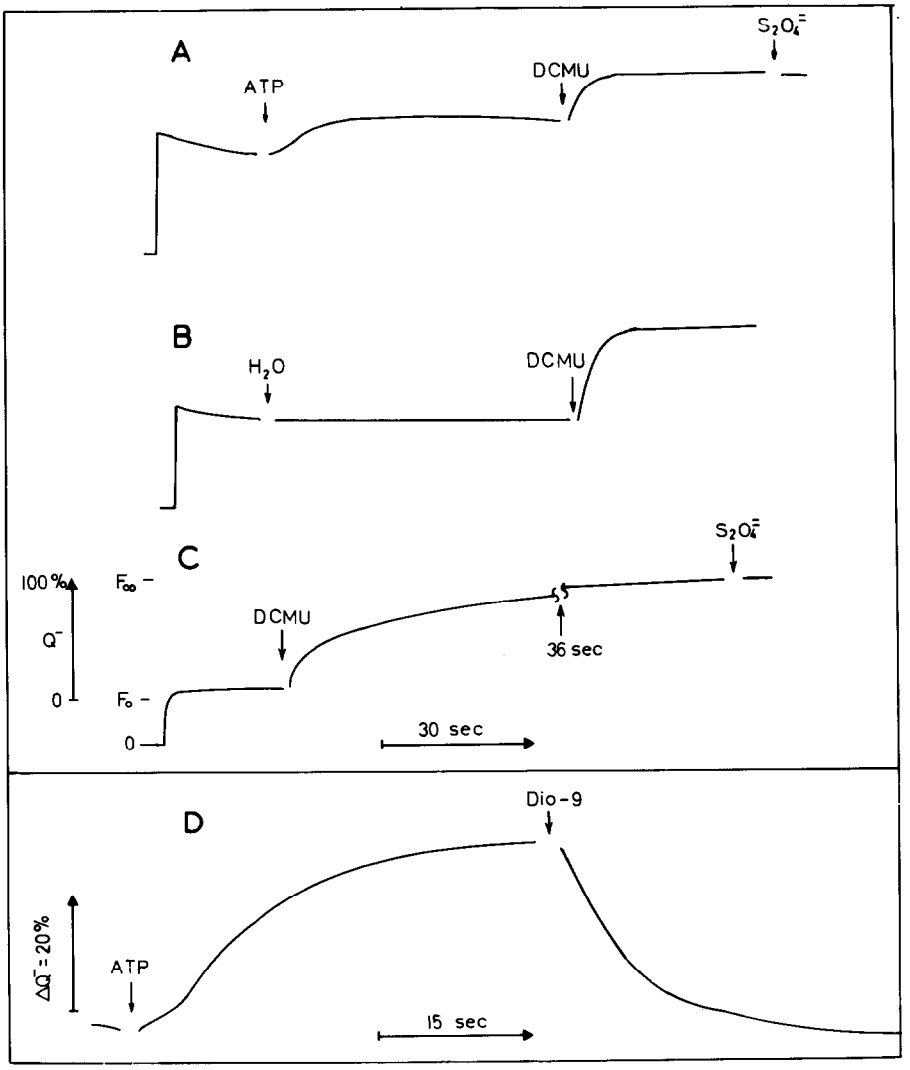

Fig. 1. ATP induced increase in fluorescence yield. Reaction conditions were as described under Materials and methods, unless otherwise indicated. A) ATP, 1 mM and DCMU, 6 μM were added where indicated. B) Water in a volume equal to that used for ATP was added where indicated. Sensitivity identical to that used in A. C) DTT and PMS omitted. DCMU, 6, μM, and a few crystals of dithionite were added where indicated. Sensitivity identical to that used in A. D) ATP, 1 mM, and D10-9 3.3 μg/ml, were added where indicated. Sensitivity 5 times higher than that employed in A, B, C.

Table 1
Conditions necessary for ATP driven rise in fluorescence yield.

Reaction conditions	Change in fluorescence yield (% of control)
Complete system	(100)
No light triggering	0
PMS omitted	−2
$MgCl_2$ omitted	6
DTT omitted	−2
H_2O added in place of ATP	0

Complete system as described under Materials and methods, except for the addition of 0.1 mM EDTA. Light-triggering was carried out in 0.18 ml reaction mixture including the chloroplasts, containing 72 μg of chlorophyll. 1.62 ml of the reaction mixture (lacking chloroplasts) was added immediately following light triggering, and 1 mM ATP 30 sec later in the usual manner. 100% refers to exchange of about 35% in the fraction of Q reduced. Negative values refer to a decrease of fluorescence yield below the steady state value observed before the addition of ATP.

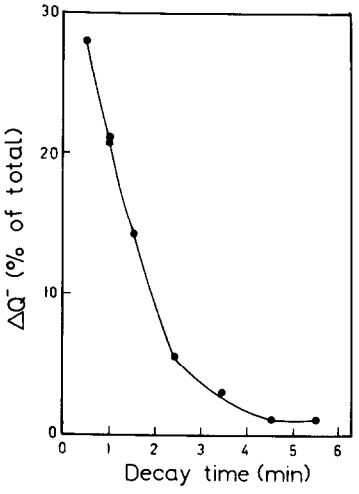

Fig. 2. Decay in the dark of the ability of ATP to induce reverse reverse electron transport. Reaction conditions as described under Materials and methods except for the time elapsed between turning off the triggering-light and the addition of ATP (decay time) which was as indicated on the abscissa.

Table 2
Substrate specificity and inhibitors of ATP driven reverse electron flow.

Compound added	In place of ATP	Before ATP
	(% of change induced by ATP)	
ADP	0	0
AMP	0	97
GTP	31*	77
UTP	−10	75
Pyrophosphate	0	78*

Reaction conditions as described under Materials and methods. When compounds were added before ATP they were added approx. 15 sec after the triggering-light was turned off, to a final concn. of 1 mM. The increase in fluorescence yield brought about by ATP (1 mM) was calculated to correspond to a change of about 37% in the fraction of Q reduced.

* In these cases the rise in fluorescence yield was slowed down considerably, and the steady state value, approached about 50 sec after the GTP or ATP addition, is recorded.

Table 3
Effect of energy transfer inhibitors, uncouplers, and electron transport inhibitors on ATP driven reverse electron transport in chloroplasts.

Compound added	Final concentration	Inhibition of ATP induced rise in fluorescence yield
Dio-9	3.3 μg/ml	89
Phloridzin	1 mM	96
NH_4Cl	3 mM	78
Atebrin	0.1 mM	106
DCMU	2.8 μM	106
NQNO	30 μM	118
BDHB	65 μM	70

Reaction conditions as described under Materials and methods. Inhibitors were added 15 sec after turning off the triggering light. In the case of the electron transport inhibitors, DCMU, NQNO and BDHB the intensity of the fluorescence exciting light was decreased to 48 ergs × cm^{-2} × sec^{-1} in order to slow down the increase in fluorescence yield due to the addition of these inhibitors. The increase in fluorescence yield brought about by ATP, was calculated to correspond to a change of about 24% in the fraction of Q reduced.

which was usually complete in about 20 sec and stable at the new level. Fig. 1B shows that no effect was seen when water replaced ATP. The changes in the redox state of Q observed in fig. 1B were quantitated by comparing with data from fig. 1C. Here the reaction mixture contained the same components as in fig. 1A or 1B, but without PMS and DTT, and was not subject to light-triggering. Instead, 60 sec preillumination with far-red light was used to restore Q to a fully oxidized state prior to fluorescence measurements [9]. The range F_0 to F_∞ (defined as that achieved with 6 μM DCMU plus a few crystals of dithionite) is linearly related to the change of Q from fully oxidized (F_0) to fully reduced (F_∞). It thus permits the calculation of the fraction of Q reduced at any time [9]. Thus in fig. 1A that fraction at the time of ATP addition was 0.44 and ATP brought about the reduction of a further 30% of Q.

In practice only the calibration (fig. 1C) was carried out at the low sensitivity necessary to cover the entire fluorescence scale. Routine experiments, such as that shown in fig. 1D, were recorded on a more sensitive scale to isolate the region undergoing the ATP dependent fluorescence yield changes.

Table 1 illustrates that the ATP induced increase in fluorescence yield was completely dependent upon achieving the light-triggered state [2, 3]. Essentially no effect was observed without light triggering, or if one of the components necessary for reaching the optimal triggered state (PMS, Mg^{2+}, DTT) was omitted.

Table 2 shows that, the increase of fluorescence yield was highly specific for ATP, with GTP a poor substitute and AMP, ADP, UTP and pyrophosphate ineffective. The table also illustrates that ADP is a potent inhibitor of the ATP induced reverse electron transport with UTP, GTP and pyrophosphate having less effect, and AMP ineffective. As previously shown [12] light triggered ATPase showed similar specificity and inhibitor sensitivity.

Fig. 2 illustrates that the light-triggered state decayed, as the time elapsed between turning off the triggering-light and the addition of ATP was prolonged. Under the conditions employed the half-time for decay was of the order of 90 sec, again similar to that previously observed for the ATPase and ATP–P_i exchange reaction [2, 3].

If the reaction was due, as the above results indicated, to reverse electron transport driven by energy supplied by the breakdown of ATP, the following compounds should block the effect: (i) energy transfer inhibitors such as Dio-9 and phloridzin which inhibit ATP hydrolysis and ATP–P_i exchange during light triggered ATPase [2, 3]. (ii) Uncouplers, which break down the high-energy state created during the breakdown of ATP, and (iii) Electron transport inhibitors, which act on the electron path between Q and the site of ATP formation.

As shown in table 3 all these predictions turned out to be true. Of particular interest is the effect of DCMU, whose exact site of action was until recently a matter of controversy with regard to what side of photosystem II was affected [13]. Our observations strongly support the generally assumed site of action as following Q in the electron transport path, and would be inconsistent with a site of action preceding photosystem II.

It may also be noted that when the addition of the energy transfer inhibitors or uncouplers noted in table 3, was made after the addition of ATP (see fig. 1D, for example) the increase in fluorescence-yield was completely reversed within a few seconds.

We believe that the results presented unequivocally demonstrate, for the first time as far as we are aware, reverse electron transport in chloroplasts. This reverse electron transport is driven by ATP through the light-triggered ATPase system. The results also support two other generally assumed, but not rigously proven sites of action: that for ATP formation which must be located between the two photosystems, and that for DCMU, which must follow Q in the electron transport chain.

References

[1] B. Petrack, A. Craston, F. Sheppy and F. Farron. J. Biol. Chem. 240 (1965) 906.
[2] C. Carmelli and M. Avron, European J. Biochem. 2 (1967) 318.
[3] K.G. Rienits, Biochim. Biophys. Acta 143 (1967) 595.
[4] C. Carmeli, FEBS Letters 7 (1970) 297.
[5] T. Bakker-Grunwald and K. Van Dam, personal communication.
[6] M. Avron and B. Chance, in: Currents in photosynthesis, eds. J.B. Thomas and J.C. Goedheer (Ad. Donker, Rotterdam, 1966) p. 455.
[7] H. Bohme and W.A. Cramer, Biochemistry 11 (1972) 1155.

[8] L.N.M. Duysens and H. Sweers, in: Studies on microalgae and photosynthetic bacteria, Japan Soc. Plant Physiol. (University of Tokyo, 1963) p. 353.
[9] S. Malkin and B. Kok, Biochim. Biophys. Acta 126 (1966) 413.
[10] M. Avron, Anal. Biochem. 2 (1961) 535.
[11] D.I. Arnon, Plant. Physiol. 24 (1949) 1.
[12] A. Bennun and M. Avron, Biochim. Biophys. Acta 109 (1965) 117.
[13] G.M. Cheniae, Ann. Rev. Plant. Physiol. 21 (1970) 467.