UNCOUPLING OF PHOTOPHOSPHORYLATION BY ATP: REMOVAL OF COUPLING FACTOR

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SUMMARY

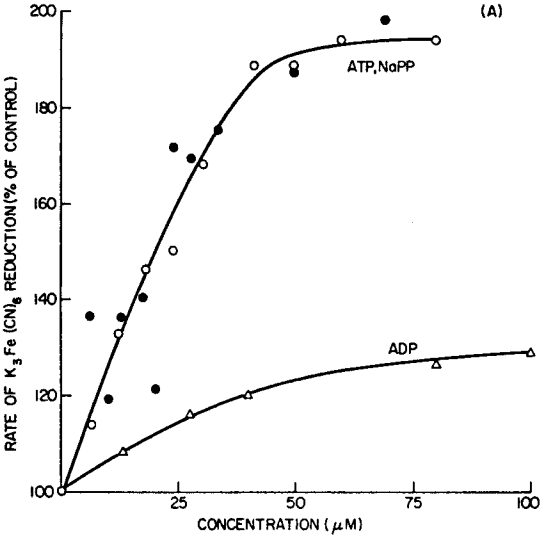
Uncoupling of photophosphorylation was observed when chloroplasts were incubated in the presence of ATP, ADP or sodium pyrophosphate under conditions of low ionic strength. Light-induced proton uptake and the 515nm absorption change were also inhibited. Mg^{++} ions reversed inhibition at Mg^{++} ATP ratios of approximately one. DCCD and chloroplast coupling factor also reversed inhibition of proton uptake suggesting that the under conditions of low ionic strength, in the absence of excess Mg^{++} ions, ATP produces uncoupling by chelating endogenous magnesium thus removing coupling factor.

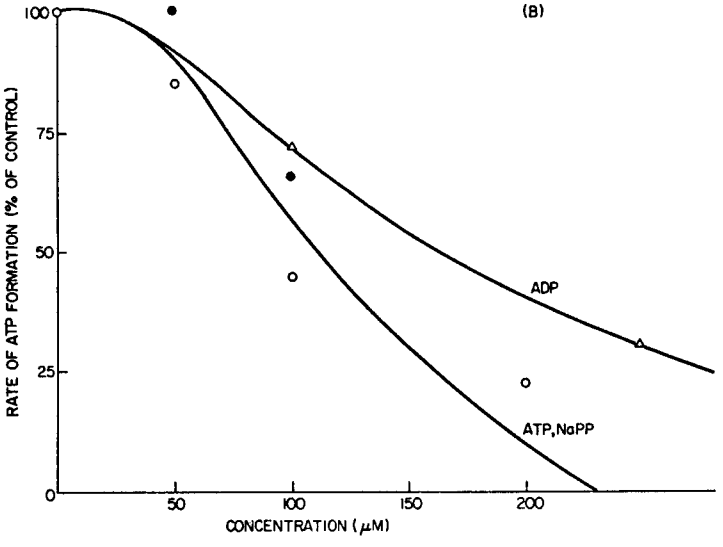
INTRODUCTION

ATP has previously been shown to inhibit photophosphorylation in chloroplasts (1-4). Two types of inhibition have been observed. One type, which occurs under conditions of high ionic strength, is accompanied by inhibition of electron transport and is thought to represent energy transfer inhibition. This type of interaction may represent a regulatory mechanism for photophosphorylation (1, 4). The second type, which has been observed under conditions of low ionic strength (2), is accompanied by stimulation of electron flow suggesting that uncoupling, not energy transfer inhibition, is occurring. We have investigated the effects of ATP on the coupling process under conditions of low ionic strength and have concluded that ATP causes uncoupling by chelating the magnesium ions required to keep the coupling factor in place.

MATERIALS AND METHODS

Chloroplasts were isolated as previously described (5). Chlorophyll concentrations were determined according to the method of Arnon (6). Rates of ATP formation and K_3 Fe(CN)$_6$ reduction were determined according to the methods of Nishimura et al (7) and Avron and Shavit (8), respectively. Light-induced proton uptake and the 515nm absorption change were determined as previously described (5).





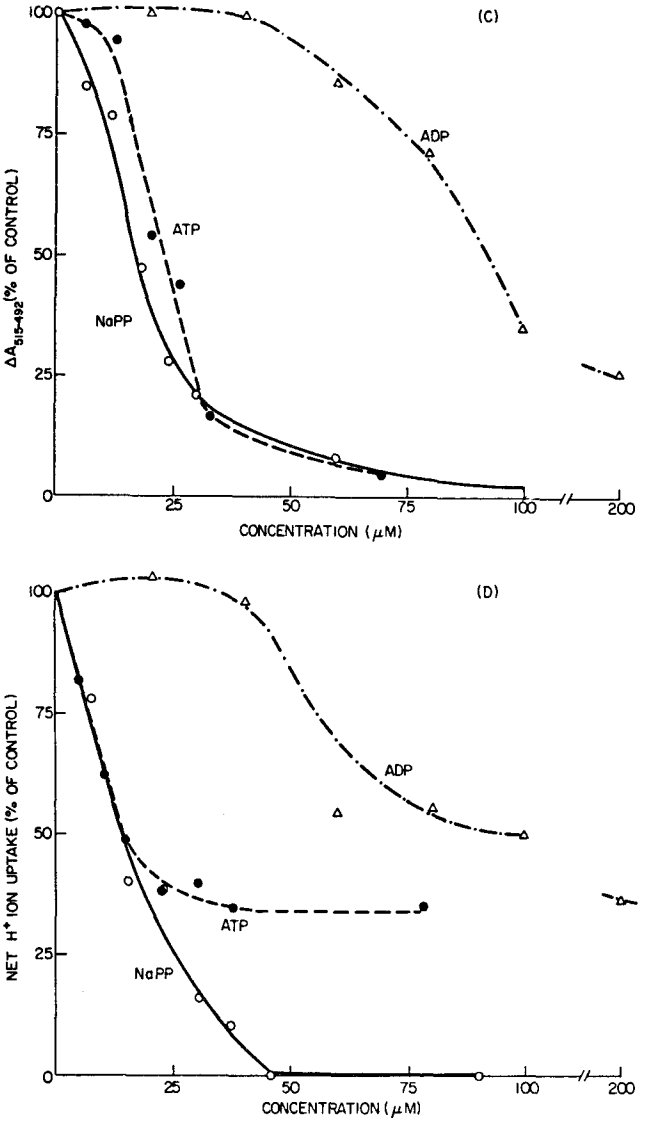


Figure 1. The effect of ATP, ADP and Na-pyrophosphate on chloroplast reactions. A) The rate of K_3 Fe $(CN)_6$ reduction was determined as a function of the concentration of the compounds indicated after chloroplasts (at 15 μg/ml chlorophyll) were illuminated for 30 sec. in a medium containing 100 mM sucrose, 0.5 mM K_3 Fe $(CN)_6$ + 0.4 mM Tris base. The pH was 8.0. The control rate of Fe $(CN)_6^{-3}$ reduction was 300 μmoles mg chlorophyll^{-1}hr^{-1}. B) The rate of ATP formation was determined as follows. Chloroplasts (at 15 μg/ml chlorophyll) were illuminated in the presence of 100 mM sucrose, 30 μM pyocyanin, 0.4 mM Tris base, 0.2 mM ADP (pH 8.0), 0.2 mM Na-phosphate (pH 8.0) and 0.2 mM $MgCl_2$ + various concentrations of the compounds indicated. The rate of ATP formation was calculated from the slope of the trace obtained after 60 sec. of illumination. The control rate of ATP

formation was 40 μmoles-mg chlorophyll^{-1}hr^{-1}. C) The $\Delta A_{515-492}$ was determined as a function of the concentration of the compounds indicated for chloroplasts illuminated in the presence of 100mM sucrose, 30 μM pyocyanin + 0.4 mM Tris base. The pH was 8.0. The control extent of the 515nm absorption change was 0.33 mg chlorophyll^{-1}. D) The extent of light-induced H^+ ion uptake was determined as for the 515nm absorption change except that white light (intensity = 5 x 10^5ergs cm^{-1}sec^{-1}) was used as the actinic light source. The control extent of H^+ uptake was 0.12 μeq-mg chlorophyll^{-1}.

RESULTS AND DISCUSSION

The effect of ATP and other nucleotides on the energy coupling process was studied under conditions of low ionic strength. The results are presented in Figure 1. It can be seen that ATP inhibition of phosphorylation was accompanied by stimulation of electron transport indicating that uncoupling, not energy transfer inhibition,was occurring. Half-maximal stimulation of DCIP reduction occurred at 20μM ATP. Higher concentrations were required to inhibit ATP formation due to inclusion of Mg^{++} ions in the reaction medium. The effect of ATP on reactions associated with high-energy states in chloroplasts was also studied. ATP was found to inhibit both light-induced proton uptake (9, 10) and the 515nm absorption change (11, 12) which has been associated with the membrane potential component of the proton-motive force (13, 14).

The effect of related compounds was also studied. ADP, like ATP, inhibited phosphorylation, proton uptake and the 515nm absorption change while stimulating electron transport. However, higher concentrations in the range of 90-100μM were required for half-maximal effects. AMP and inorganic phosphate showed only slight effects at higher concentrations (~ 1mM). However, sodium pyrophosphate was as effective as ATP on a concentration basis. Cytosine, guanidine, and uridine nucleotides were found to be as effective as their adenosine analogs. The pattern which emerges is that the most effective

compounds are those with the highest binding constants for Mg^{++} ions (15, 16). These results suggest that, under conditions of low ionic strength, ATP causes uncoupling in a manner similar to that of EDTA (17) by removing the Mg^{++} ions required for attachment of coupling factor to the membranes.

If this is so, the following should be true. First, addition of $MgCl_2$ should reverse inhibition and furthermore, the extent of reversal should be dependent on the Mg:ATP ratio rather than the absolute Mg^{++} ion concentration. The results presented in Figure 2 show that, at two different ATP concentrations, 100% reversal of inhibition of the 515nm absorption change occurred at Mg:ATP ratios slightly greater than one which verifies this prediction.

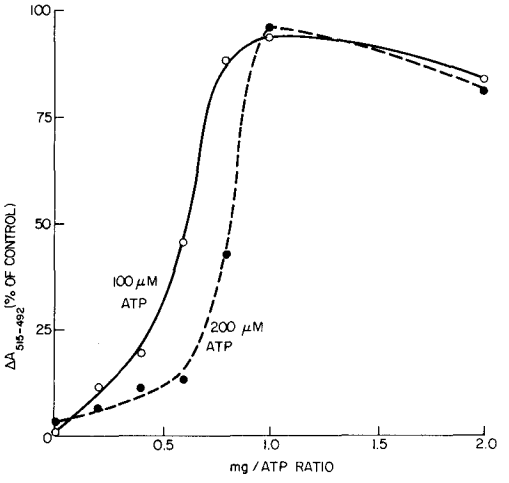


Figure 2. The effect of the Mg:ATP ratio on ATP-induced inhibition of the 515nm absorption change. The 515nm absorption change was determined as a function of $MgCl_2$ concentration in the presence of 100 and 200 μM ATP respectively. The control value of the 515nm absorption change observed in the absence of ATP was 0.36 mg chlorophyll^{-1}.

Second, the energy transfer inhibitor dicyclohexyl carbodiimide (DCCD) (17) has been shown to restore light-induced proton uptake in chloroplasts depleted of coupling factor by EDTA treatment. If ATP acts in an analogous

Table I
Reversal of ATP, ADP and Pyrophosphate Inhibition of Light-Induced H^+ Ion Uptake by DCCD and Chloroplast Coupling Factor

	Net H^+ ion Uptake (μeq-mg chlorophyll^{-1})	
Additions	-DCCD	+100 μM DCCD
Expt. 1.		
None	0.105	0.145
ATP (100 μM)	0.033	0.075
ADP (250 μM)	0.026	0.087
Na-PP_i (100 μM)	0.030	0.070
Expt. 2.	-CF_1	+CF_1
None	0.097	0.115
ATP (100 μM)	0.037	0.105

Light induced proton uptake was determined as for Fig. 1. Coupling factor (CF_1) was obtained from the supernatant of EDTA treated chloroplasts (16). An aliquot containing 1.5 mg protein was added where indicated.

manner, DCCD should restore proton uptake. The results presented in Table 1 show that DCCD reversed inhibition by ATP, ADP and pyrophosphate.

Third, if ATP-uncoupling is due to removal of coupling factor, the addition of coupling factor contained in the supernatant of EDTA-treated chloroplasts should reverse it. The results presented in Table 1 show that this is so.

In conclusion, the results indicate that, under conditions of low ionic strength, ATP can cause uncoupling of photophosphorylation by chelation of endogenous Mg^{++} resulting in removal of coupling factor.

ACKNOWLEDGMENTS

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