ON THE INTERACTION OF ATP WITH THE PHOSPHORYLATION SYSTEM IN CHLOROPLASTS

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Received 5 August 1970
Revised version received 24 September 1970

1. Introduction

The process of photophosphorylation in chloroplasts has long been regarded as irreversible [1]. However, more sensitive tests made with exchange reactions have shown significant reversal of various steps [2, 3]. Regarding the metabolic significance of the reversal, however, the reaction is still considered essentially irreversible.

In a study of the effects of phophorylating reagents on ferricyanide reduction, Avron et al. [4] observed that ATP, at low concentrations, can partially inhibit electron flow rate. The degree of inhibition of electron flow by ATP was not affected in the presence of Mg^{2+} and/or Pi. We recently reported that the addition of salt or ATP to the acid stage, inhibited the synthesis of ATP in the acid–base phosphorylation reaction [5].

In this communication we wish to report the inhibition, of ATP synthesis and the coupled electron flow, in the light dependent photophosphorylation reactions as well as in the acid–base phosphorylation reaction. The inhibition by ATP of these reactions, together with the lack of inhibition by ATP in the presence of an uncoupler or an energy transfer inhibitor, suggest that ATP affects a high-energy state or intermediate of photophosphorylation.

2. Materials and methods

Chloroplasts were isolated from fresh market lettuce leaves by standard procedures [6]. Ferricyanide reduction and ATP formation were assayed as described [7, 8]. ATP formation induced by acid–base incubation of chloroplasts was assayed as described [9]. Solutions of ATP (from Sigma) at appropriate pH's were added to the reaction mixtures of the acid–base or the light dependent phosphorylation reactions. Light intensity was 160,000 lux of white light. FCCP* was a generous gift from Dr. W.W.Prichard, E.I.Du Pont de Nemours & Co., Wilmington, Delaware, U.S.A.

3. Results

The inhibition of the acid–base induced ATP synthesis by ATP is given in fig. 1. It is noteworthy that when ATP was added to the acid incubation mixture it was present in this reaction mixture at the concentration indicated, and at half this concentration in the subsequent basic stage of the reaction. When added to the basic stage only it was present solely during this stage of the reaction. No effect was observed when ATP was added to the basic reaction mixture at concentrations identical to those of the acid reaction mixture, where it strongly inhibited the synthesis of ATP. Hence, it is suggested that ATP acts by affecting the "high-energy state" formed during the acid incubation. This experiment also excludes the possibility that the inhibition may be due to simple dilution of the label with unlabelled Pi derived from ATP.

ATP synthesis was also inhibited by other nucleoside triphosphates such as UTP, GTP and CTP. None

* *Abbreviations:* FCCP: carbonylcyanide *p*-trifluoromethoxy phenylhydrazone; CHL: chlorophyll.

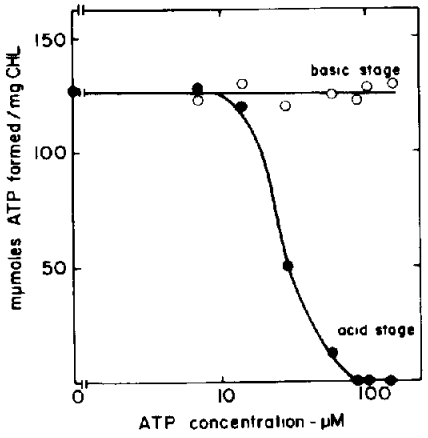

Fig. 1. The effect of ATP on the dark, acid–base induced ATP formation. The procedure for ATP formation induced by acid–base incubation of chloroplasts consisted of two successive stages, both carried out at 0°: (a) isolated chloroplasts were exposed to an acid pH and (b) the pH was raised in the presence of ADP, Pi and Mg^{2+} ions. The reaction mixture in stage (a) was contained in a volume of 1.5 ml at pH 4.0 as follows: succinic acids, 10 mM; chloroplasts containing 83 μg chlorphyll and ATP where indicated. After 30 sec, the acidified chloroplasts were injected into a reaction mixture containing in a volume of 1.5 ml the following components in μmole: tris-HCl, 90; ADP, 0.2; Pi, (containg 2×10^6 cpm of ^{32}P) 1.0; $MgCl_2$, 5.0. Final pH was 8.0. The phosphorylation reaction was stopped after 60 sec by adding 0.3 ml of 30% TCA. $AT^{32}P$ was assayed as described [8]. ATP concentrations are given for each stage. The results marked "acid stage" (•) were obtained in this way. Those marked "basic stage" (○) were obtained similarly, except that there was no ATP at stage (a). The ATP, as indicated, was present in the second 1.5 ml of reaction mixture.

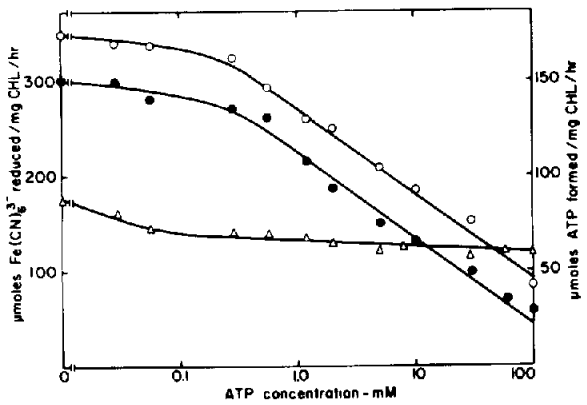

Fig. 2. The effect of ATP on electron flow and phosphorylation with ferricyanide. Reaction mixture for coupled electron flow and phosphorylation contained in a volume of 3 ml the following components in μmoles: KCl, 150; Tricine–NaOH, 10; $MgCl_2$, 5.0; ADP, 2.0; Pi (containing 1×10^6 cpm of ^{32}P) 2.0; $K_3Fe(CN)_6$, 1.0 and chloroplasts containing about 60 μg chlorophyll. The pH was 7.8. After illumination for 60 sec, reaction was stopped by adding 0.3 ml of 30% TCA. In reaction mixtures of the basal electron flow assay, ADP, Pi and $MgCl_2$ were omitted. (○) coupled ferricyanide reduction; (•) ATP formation; (△) basal ferricyanide reduction.

of the corresponding dinucleotides nor AMP was inhibitory. Addition of equimolar amounts of ADP and ATP to the acid stage had no effect on the degree of inhibition by ATP. Fifty percent inhibition of the synthesis of ATP in this reaction was attained at about 3×10^{-5} M ATP.

The light dependent ATP formation with ferricyanide or *N*-methylphenazonium methosulphate was also inhibited by addition of ATP. Moreover, ATP inhibited the coupled electron flow and the accompanying phosphorylation rate as shown in fig. 2. Fifty percent inhibition was attained at about 3×10^{-3} M, a value about 100-fold higher than in the case of the acid–base induced ATP synthesis. However, even at these high concentrations of ATP, the basal electron flow rate (e.g., without ADP, Pi and Mg^{2+} ions) was only slightly inhibited. This led us to investigate the effect of ATP on electron flow uncoupled from phosphorylation, in the presence of an uncoupler such as FCCP. As indicated in fig. 3, the uncoupled electron flow is insensitive to inhibition by ATP.

Another tool used to inhibit the coupled electron flow rate was the compound Dio-9, an energy transfer inhibitor. This compound is known to act as an energy transfer inhibitor at certain concentrations [10] and may act as an uncoupler at higher concentrations*. In the range of concentrations at which Dio-9 inhibits the coupled electron flow rate and the accompanying ATP formation, with ferricyanide as electron acceptor, the ATP inhibitory action on the coupled electron flow was almost abolished (fig. 4).

* Personal communication, S.J.D.Karlish and M.Avron; and unpublished experiments.

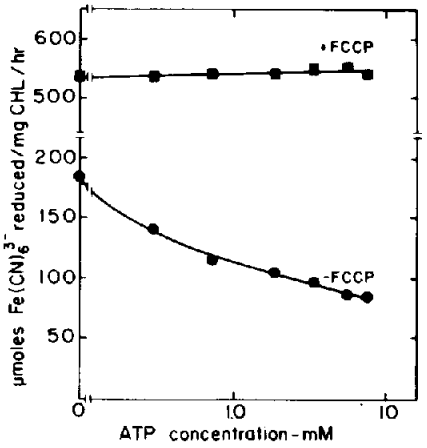

Fig. 3. The effect of ATP on ferricyanide reduction in the presence of FCCP. Reaction mixtures and assay conditions as described in Methods and in fig. 2. FCCP concentration was 10^{-6} M.

We also investigated the effect of ADP, Pi and $MgCl_2$ on the inhibition of electron flow rate by ATP. There appears to be no competition between each of the phosphorylating agents and ATP up to ratios of 2:1.

4. Discussion

Among the plausible modes of action for ATP inhibition of ATP synthesis and, moreover, the electron flow rate, in photophosphorylation reactions in chloroplasts, is the proposed ATP interaction with a high-energy intermediate or state which originates in electron flow and ultimately participates in ATP formation.

ATP may affect the pH gradient established upon illumination, or, in the case of the acid–base induced reaction, diminish the amount of succinic acid penetrating the chloroplasts. This could be a direct effect on the permeability of the chloroplast membrane or may be due to a change in the volume of the particles. An experiment was performed in which ATP was added at different intervals of the acid stage period. The inhibition by ATP, in this case, when added after the chloroplasts had already been exposed to the acid pH, was smaller than when ATP was present during the complete acid stage period. This experiment suggests that the permeability of the chloroplast membrane to succinic acid may be an important factor in the mechanism of inhibition by ATP.

The suggested effect of ATP on a high-energy stage or intermediate seems to be supported by: (a) the presence of ATP being required during the activation stage in the acid–base phosphorylation reaction; (b) the inhibition by ATP of the coupled rather than the basal rate of electron flow; (c) the inhibition being released by an uncoupler, and (d) the inhibition of the electron flow rate by ATP being diminished in the presence of Dio-9. ATP would therefore appear to act as an energy transfer inhibitor, and a very interesting one indeed in view of its being a product of the phosphorylation reaction.

Further investigation is required to assess whether ATP exerts its effect by shifting the equilibrium of the forward phosphorylation reaction or through an effect on the permeability of the chloroplast membrane. The lack of competition of ATP with ADP, Pi and Mg^{2+} ions seems to suggest the latter.

The inhibition by ATP of the coupled rate of electron flow, even below that of the basal electron flow rate, may be an indication of the type of electron flow participating during photophosphorylation reaction [11]

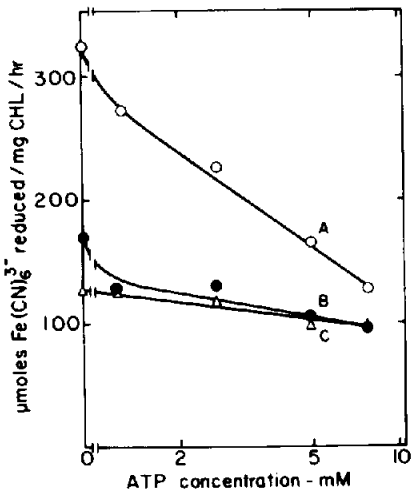

Fig. 4. The effect of ATP on ferricyanide reduction in the presence of Dio-9. Reaction mixtures and assay conditions as described in Methods and in fig. 2. Dio-9 concentrations in μg/ml were: A) nil; B) 4; C) 7; In the absence of added ATP, 4 and 7 μg/ml of Dio-9 inhibited ATP formation 65 and 88, respectively.

Acknowledgements

We are grateful to Mrs.L.Zuckerman for competent technical assistance and to Mrs.Cynthia Bellon for editing the manuscript.

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