SYNTHESIS OF LABELLED ATP FROM ADP AND LABELLED PYROPHOSPHATE BY SPINACH CHLOROPLASTS

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1. Introduction

Recently several reports demonstrated a unique role of pyrophosphate in photophosphorylation and photosynthetic CO_2 fixation of several organisms [1-6]. According to Baltscheffsky [3, 4], pyrophosphate is linked to the still unknown phosphorylated intermediate X ~ P (fig. 1). Bacterial chromatophores, in the absence of the phosphate acceptor ADP, produce pyrophosphate, thus converting light energy into the pyrophosphate bond. Besides the well known exchange reaction between ATP and P_i [7-11], an isotope exchange reaction between ATP and pyrophosphate was also demonstrated [12, 13]. According to the scheme of phosphorylation in fig. 1, it can be assumed that similar to the action of light or of an acid-base transition, pyrophosphate can build up the energy-rich intermediates of the phosphorylation chain.

The present paper gives evidence of this hypothesis by demonstrating a synthesis of labelled ATP from ADP and labelled pyrophosphate in the dark.

2. Methods

Chloroplasts were prepared according to the procedure of Whatley and Arnon [14]. Chloroplast fragments were incubated in the dark in a medium containing chloroplast fragments (400 μg chlorophyl), Tris-buffer pH 8 (33 mM), NaCl (16 mM), ^{32}P-pyrophosphate (0.3 mM), $MgCl_2$ (3.3 mM), ADP (3.3 mM), DTT (6.6 mM), total radioactivity per sample equal to 2×10^6 cpm, total volume 2.8 ml. The reaction was stopped by addition of TCA (final concentration 2%) and the precipitated proteins removed by centrifugation. Aliquots of the supernatant were chromatographed on thin-layer chromatography (cellulose (CAMAG, Switzerland)), 0.5 m thickness; solvents: (a) saturated ammoniumsulfate 80, 1 m sodiumacetate 18, isopropanol 2, (b) after Aurenge et al. [15]: H_2O 30 ml, ethanol 96% 35 ml, isobutanol 15 ml, isopropanol 20 ml, ammonia 25% 0.4 ml, trichloroacetic acid 5 g. Nucleotides were detected in the UV, phosphate and pyrophosphate after spraying with molybdate [16]. Radioactivity was detected by autoradiography, the thin-layer plates were exposed to Agfa-Gevaert X-ray film (DW) and developed after suitable intervals.

3. Results

As demonstrated in the chromatograms (fig. 2), in contrast to the experiments on ATP/P_i exchange,

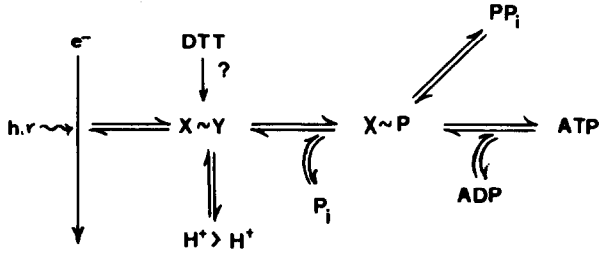

Fig. 1. Proposed mechanism of phosphorylation. Activation of the exchange reaction between ATP and P_i by light, acid base transition or DTT. Pyrophosphate connected to X ~ P.

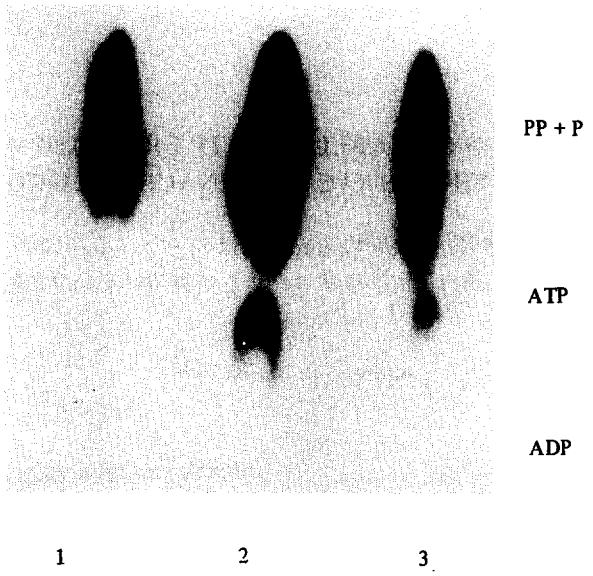

Fig. 2. Chromatogram of the labelled products of the synthesis of ATP from ADP and pyrophosphate. Reaction medium in the text. (1) Reaction time: 0 min. (2) Reaction time: 30 min in the dark after illumination of 2 min, addition of ADP after illumination. (3) Reaction time: 30 min in the dark, no preillumination.

DTT and the triggering by light are not essential for an incorporation of ^{32}P-phosphate from pyrophosphate into ADP. The reaction proceeds approximately linearly with time over 60 minutes and seems to be proportional to the amount of chloroplasts in the reaction mixture. As well known for similar reactions described for mitochondria and chloroplasts, magnesium is also essential for the reported synthesis of ATP from ADP and pyrophosphate. ADP but not AMP can act as phosphate acceptor from pyrophosphate. The addition of inorganic phosphate does not reduce the amount of labelled phosphate incorporated into ATP, thus excluding any equilibrium between inorganic phosphate and intermediary products of the ATP synthesis from pyrophosphate and excluding therefore a possible labelling of the ATP by the action of an exchange reaction with inorganic phosphate together with the high activity of the chloroplast-myokinase. Experiments were carried out with different inhibitors of phosphorylation reactions, including desaspidin 10^{-7} m, gramicidin 10^{-7} m, phlorizin 10^{-5}, oligomycin 10^{-5} and valinomycin 10^{-5}: The described synthesis of ATP from ADP and pyrophosphate showed a sensitivity to the inhibitors tested equivalent to that of normal phosphorylation.

4. Discussion

The reaction described can be written in two steps:

(1) pyrophosphate + enzyme $\rightleftharpoons$ enzyme $\sim$ P + P_i

(2) enzyme $\sim$ P + ADP $\rightleftharpoons$ enzyme + ATP.

It may be concluded from the studies of inhibitors that the ATP formation with pyrophosphate and the ATP/P_i exchange reaction exhibit similar properties; it is therefore probable, that the reaction (2) above is part of the phosphorylating pathway in chloroplasts. In contrast to light induced phosphorylation, there is no need for electron transport; light and addition of the electron carrier PMS do not enhance the formation of ATP under the described conditions. Furthermore the building up of a high energy membrane state, either by light or by an acid base transition as found necessary for the ATP/P_i exchange [7, 9] and for the ATPase [12, 18], is no prerequisite for an ATP synthesis from ADP and pyrophosphate. A thiol reagent, activating ATP/P_i exchange and ATP hydrolysis, has no remarkable effect on the ATP synthesis with pyrophosphate.

Of special interest may be the intermediate of the reaction, described as enzyme ~ P in the reaction scheme above. The compound enzyme ~ P could be identical with the phosphorylated high energy intermediate X ~ P of fig. 1. Unfortunately little is known of the nature of the energy-rich intermediates of phosphorylation. Decay times of these intermediates differ markedly, X_E of Neumann and Jagendorf [19], as measured by pH change, has a half life of 2 to 30 seconds depending on the pH, X_E of Bachofen and Specht [9] and Specht and Bachofen [11] a half life time up to 30 min, as measured by the ability to catalyse the ATP/P_i exchange reaction. At the moment it is not possible to estimate the decay time of X ~ P produced by pyrophosphate. Methods for characterizing the reaction of ATP synthesis from ADP and pyrophosphate are in preparation.

5. Summary

A reaction is described, by which chloroplasts synthesize labelled ATP from ADP and pyrophosphate. The reaction proceeds in the dark, is dependent on Mg ions and ADP and is inhibited by known inhibitors of photophosphorylation.

Acknowledgement

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