EXCHANGE REACTION BETWEEN ATP AND LABELLED PYROPHOSPHATE BY ISOLATED SPINACH CHLOROPLASTS

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1. Introduction

As is well known the high energy intermediate X~P in photophosphorylation which leads to the formation of ATP can be built up by light [1,2] or acid-base transition [3,4]. This has led to the hypothesis that the energy reservoir may be identical with a proton-gradient across the grana membrane [5]. Recently several reports have referred to the role of P~P in photosynthesis. Vose and Spencer [6] found in photosynthetic CO_2 fixation, that P~P could replace ATP (see also [7,8]); H. Baltscheffsky [9] showed the formation of P~P instead of ATP in the absence of nucleotides by photosynthetic active bacterial chromatophores and M. Baltscheffsky [10] pointed to the close linkage of P~P to the phosphorylation step between cyt. b and cyt. c. A synthesis of ATP from ADP and P~P in chloroplasts has recently been demonstrated [11] where no other energy donor besides P~P was needed (reaction in the dark without DTT).

According to previous reports a scheme of photophosphorylation can be constructed [12], where P~P is able to build up one of the energy-rich intermediates of phosphorylation (fig. 1).

The energy and thiol-group dependent exchange reaction between ATP and P_i is thought to refer to one of the last steps in phosphorylation [13-17], and consistent with this the present paper shows the possibility of inducing an exchange between ATP and P~P without additional energy sources, giving further evidence of the close relationship of P~P to X~P.

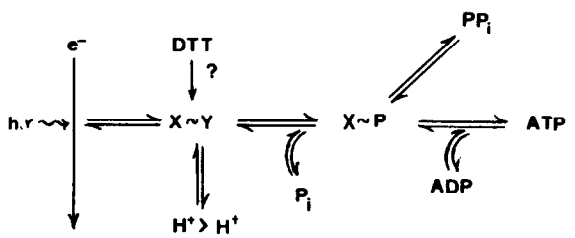

Fig. 1. Proposed mechanism of phosphorylation. Activation of the exchange reaction between ATP and P_i by light, acid-base transition or DTT. Pyrophosphate connected through X ~ P.

2. Methods

Chloroplasts were prepared by the procedure of Whatley and Arnon [18]. The reaction medium contained: Chloroplast fragments (400 μg chlorophyll/2.8 ml total volume), Tris-buffer pH 8 (33 mM), NaCl (16 mM), $MgCl_2$ (3.3 mM), DTT (6.6 mM), ^{32}P-pyrophosphate (0.3 mM, about 3×10^6 cpm per sample), ATP (3.3 mM). The reaction was stopped by addition of TCA (final concentration 2%) and the precipitated proteins were removed by centrifugation. Aliquots of the supernatant were chromatographed by thin-layer chromatography (for methods see [11]) or the labelled nucleotides were estimated according to Avron [19] in a liquid scintillation counter.

3. Results

As reported before [15] there is a small endogenous

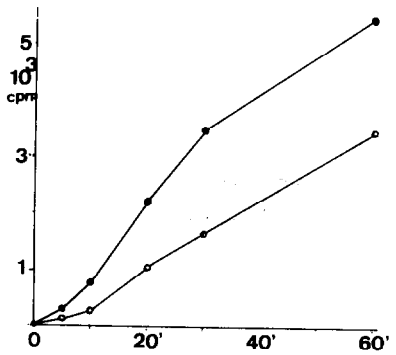

Fig. 2. Time course for ATP-P_i exchange reaction. • without preincubation with pyrophosphate; ○ with preincubation (10 min) with pyrophosphate (3.3 mM).

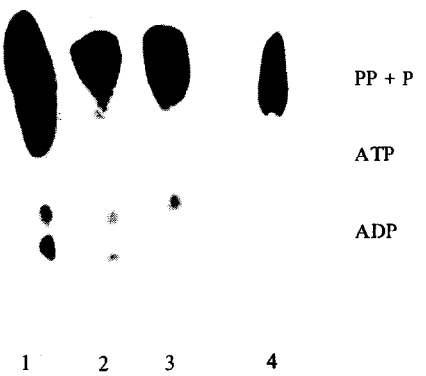

Fig. 3. Chromatogram of the products labelled by the exchange reaction between ATP and ^{32}P-pyrophosphate. Reaction medium in the text. (1) 2 min light. Reaction time in the dark: 30 min. (2) Reaction in the dark. Reaction time: 30 min. (3) Reaction in the dark with addition of 16 mM phosphate. Reaction time: 30 min. (4) Reaction time: 0 min.

DTT dependent ATP-$^{32}P_i$ exchange in chloroplasts in the dark under the conditions described. This reaction shows different kinetics compared to the light-triggered ATP-P_i exchange reaction in giving slow but nearly linear rates for long reaction times (> 60 min). If the mechanism proposed above (fig. 1) is right, preincubation with P~P should reduce the rate of ATP-P_i exchange due to some kind of equilibrium between X~P, P~P and the nucleotides. Fig. 2 gives clear evidence for this opinion (measured counts give the total radioactivity of all labelled nucleotides!).

In the chromatogram (fig. 3) the results of an exchange reaction between ATP and ^{32}P-pyrophosphate without added phosphate are demonstrated. This reaction is stimulated by light-triggering (column 1; for comparison in column 4 the reaction time 0 min is shown) but takes place in the dark too (column 2). The addition of large amounts of unlabelled phosphate (16 mM) does not significantly reduce the amount of labelled ATP (column 3) thus excluding the exchange between ATP and $^{32}P_i$ released from ^{32}P~P by strong pyrophosphatase activity.

In contrast to the ATP synthesis reaction with P~P [11] the exchange reaction under all the conditions described above (except the control) shows on the chromatogram significant but variable amounts of labelled ADP besides ATP (strongest labelling in the light-triggered exchange with P~P). This could be due to a myokinase activity (unpublished results) activated under the conditions of the exchange reaction where an equilibrium between ATP, AMP and ADP leads to the observed effect. Moreover added AMP lowered the amount of labelled ATP. Column 3 on the chromatogram, with the addition of unlabelled P_i, points to this interpretation in showing merely a small spot of labelled ADP presumably due to an interaction between ADP and P_i rather than ATP and P_i under these conditions.

Additional experiments showed the necessity of Mg^{2+} and DTT for the reaction described and a sensitivity to some inhibitors of photosynthesis (full inhibition: phlorizin 10^{-3}M, oligomycin 10^{-4}M, desaspidin 10^{-7} to 10^{-6}M) similar to that of ATP synthesis from ADP and P~P [11].

4. Discussion

Experiments described above give further evidence that the energy of the pyrophosphate bond can be directly used in phosphorylation reactions. The first step of the reaction [11]:

$$^{32}\text{pyrophosphate} + \text{enzyme} \rightleftarrows \text{enzyme-}^{32}\text{P} + {}^{32}\text{P}$$

is also part of the exchange reaction. A second step will lead to the labelling of ATP:

$$\text{enzyme-}^{32}\text{P} + \text{ATP} \rightleftarrows \text{enzyme-P} + \text{AT}^{32}\text{P}.$$

Bachofen et al. [11] concluded from their experiments, that ATP formation from P ~ P needs no electron-transport (no stimulation by light and PMS); and no formation of a high energy membrane state is necessary. Furthermore thiol reagents have little influence on the reaction. On the contrary the ATP exchange reactions with P ~ P need DTT and light shows a stimulating effect. Additionally the different labelling of ADP under various conditions points to a more complex situation. There is a high myokinase activity and the equilibrium of this reaction has a great effect on the quantitative relations of the various nucleotides to each other. On the other hand the amount of P_i in the medium affects the labelling of ADP; this is also true for the exchange reaction between ATP and P_i in the absence of pyrophosphate.

Consequently we have to admit that the interactions between X ~ P, P ~ P, P_i, ATP and ADP are not yet quite clear concerning the mechanism of the ATP exchange reaction, and fig. 1 merely gives a working hypothesis. For further investigations a method allowing a quick quantitative determination of each nucleotide and phosphate present in the reaction mixture would be useful.

5. Summary

In chloroplasts P ~ P can directly be used for an exchange reaction with ATP; furthermore an exchange with ADP also takes place. These reactions are dependent on DTT, stimulated by light and sensitive to some inhibitors of photophosphorylation.

Acknowledgement

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