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Received October 23rd, 1962

PN 10019

Enzymic formation of adenosine triphosphate with acetyl phosphate as donor in a yeast extract

When studying the polyphosphate synthesis by polyphosphate kinase (ATP: polyphosphate phosphotransferase, EC 2.7.4.1) in a yeast extract using ^{32}P-labelled acetyl phosphate, ADP, and a preparation of bacterial acetate kinase (ATP: acetate phosphotransferase, EC 2.7.2.1) as ATP-forming system (*cf.* MUHAMMED[1]), it was found that polyphosphate synthesis proceeds almost at the same rate when the acetate kinase was omitted from the reaction mixture. This observation seems to imply that our yeast extract contains an enzyme which catalyzes the transfer of a phosphate residue from acetyl phosphate to ADP.

A preparation of this enzyme can be obtained by treating a suspension of 3 g well-washed pressed baker's yeast in 30 ml isotonic phosphate buffer (pH 7.4) containing 0.05 M cysteine, in the cell disintegrator of MERKENSCHLAGER *et al.*[2] for 3 min. The resulting mixture is diluted with the same amount of the buffer solution and then centrifuged at 20 000 × *g* for 1 h at 4°. The supernatant contains the enzyme; it can be dialyzed against the buffer solution containing cysteine without loosing its activity. Its protein content is 15–20 mg/ml, measured according to WARBURG AND CHRISTIAN[3]. An approximately 3-fold concentration of the enzyme can be achieved by saturation with ammonium sulfate to 60% of the solution and removal of the inactive precipitate.

Enzyme activity is measured by incubating a mixture of 10 μmoles labeled acetyl phosphate (prepared according to KORNBERG *et al.*[4]) in 0.1 ml water, 10 μmoles ADP in 0.1 ml water, and 0.3 ml of the buffered enzyme preparation for 15 min. Then the reaction is stopped by adding 1 ml 7% $HClO_4$. For the removal of polyphosphate, formed by the polyphosphate kinase present in the preparation, 1.5 ml of a 0.1% albumin solution are added. After 10 min cooling in ice-water the mixture is centrifuged for 10 min at 6000 × *g* and 4°. To the supernatant 10–20 mg Norite charcoal are added and the mixture is shaken for 30 min. The Norite is then centrifuged off (6000 × *g* at 4°) and washed with distilled water until the activity of the water is negligible.

For routine measurements the activity of the Norite suspension can be measured directly in a liquid β-counter (M2H, 20th Century Electronics Ltd.). For more exact determinations, it is necessary to elute with 10% pyridine; the activity is measured in the eluate.

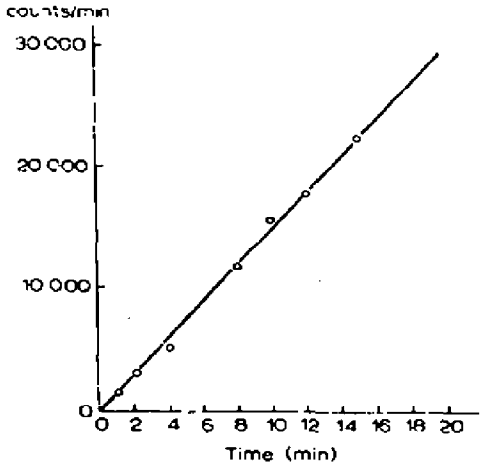

Fig. 1. Time dependence of the formation of Norite-adsorbable material (TAM) from acetyl [^{32}P]phosphate and ADP. The assay conditions are described in the text.

Under the conditions described, a rapid formation of a radioactive Norite-adsorbable substance takes place (Fig. 1). The enzyme reaction shows a pH optimum at about 7.1 (Fig. 2). Cysteine has a marked stabilizing effect on the enzyme. No

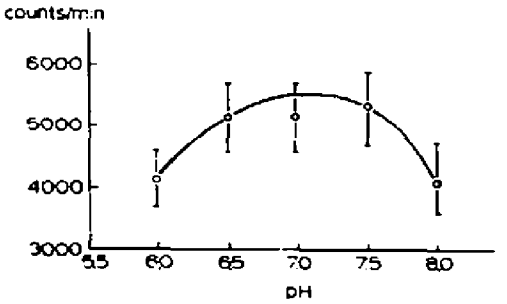

Fig. 2. Effect of H^+ concentration on the velocity of the enzyme reaction. Tris–malcate buffer of varying pH was used.

requirement for Mg^{2+} could be observed; fluoride does not inhibit at a concentration of 10 mM (Table 1). K^+ and NH_4^+ show some activating effects. Very high concentrations of acetyl phosphate inhibit. A determination of K_m for acetyl phosphate gave an approximate value of 2.3 mM.

In order to establish the nature of the reaction product, the pyridine eluate was freed of the pyridine and then analyzed by paper chromatography (propanol · NH_3–water, 6 : 3 : 1) and by electrophoresis (citrate buffer (pH 3.2) 2000 V, no cooling). With both methods, the reaction product migrated together with authentic ATP.

Experiments to establish the reverse reaction, *i.e.* the formation of acetyl phosphate from ATP and acetate, have failed so far. No reaction could be obtained with the classical hydroxamic acid method[5] and an attempt to demonstrate the formation of acetyl phosphate by coupling the reaction with the action of phosphate

TABLE I

FORMATION OF ^{32}P-LABELLED NORITE-ADSORBABLE MATERIAL FROM ACETYL [^{32}P]PHOSPHATE AND ADP

Complete system contains 4.5 mg protein (from undialyzed enzyme extract); 30 μmoles potassium phosphate buffer (pH 7.4); 3 μmoles $MgCl_2$; 10 μmoles acetyl[^{32}P]phosphate (80 000 counts/min); 10 μmoles ADP; 15 μmoles cysteine, in a total volume of 0.5 ml. Incubation at 37° for 15 min.

Incubation conditions	*Activity of product (counts/min)*
Complete system	7410
Same without enzyme	51
Same without ADP	1958
Same without Mg^{2+}	7541
Same plus fluoride (30 μmoles)	7451

transacetylase (EC 2.3.1.8) in the presence of arsenate did not give unequivocal results.

The results presented in this paper indicate the existence of an enzyme in yeast which can use acetyl phosphate for ATP synthesis and therefore should be classified as an acetate kinase. This is somewhat surprising because several earlier authors (*cf.* OCHOA AND STERN[6]) reported that no enzyme of this specificity exists in yeast. At that time, however, the hydroxamic acid method was used exclusively for detecting acetate kinase activity.

Further work on the characterization and purification of the enzyme and an investigation whether it is identical with other enzyme entities, *e.g.* carbamate kinase (EC 2.7.2.2) (*cf.* GRISOLIA *et al.*[7,8]) are in progress.

This study was supported in part by a grant from the Ludwig Boltzmann-Gesellschaft, Vienna. We are grateful to Dr. A. MUHAMMED (Lahore, West Pakistan) for assistance and valuable discussions in the early phase of this work, and to Messrs. C. F. Boehringer und Söhne GmbH, Mannheim (Germany) for a generous gift of crystalline phosphate transacetylase.

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Received October 26th, 1962

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