Short Communications

Specificity of pyruvate kinase

Previous reports have indicated that pyruvate kinase is a relatively non-specific enzyme capable of catalyzing the transfer of phosphate from PEP to a wide variety of nucleoside diphosphate acceptors[1,2]. In the present studies, attempts were made to utilize this enzyme for the assay of UDP. The rate of phosphate transfer to nucleoside diphosphate was followed by coupling reactions II (pyruvate kinase) and III (lactic dehydrogenase) and measuring the change in absorbance at 340 mμ indicative of the oxidation of DPNH. Preliminary results indicated that the assay for UDP was variable and was dependent on the source of the nucleotide, and the age and purity of the enzyme preparations employed for assay purposes.

$$\text{I. Nucleoside-P-P} + \text{ATP} \rightleftharpoons \text{Nucleoside-P-P-P} + \text{ADP}$$
$$\text{II. Purine-R-P-P} + \text{PEP} \longrightarrow \text{Purine-R-P-P-P} + \text{Pyruvate}$$
$$\text{III. Pyruvate} + \text{DPNH} \longrightarrow \text{Lactate} + \text{DPN}$$

Since the possibility remained that reaction I (nucleoside diphosphokinase[3]) was being coupled to reactions II and III, the specificity of pyruvate kinase was examined.

Pyruvate kinase was prepared as described and twice crystallized[4]. The crystalline preparation was diluted 1 to 50 before use giving a protein concentration of 8 μg per aliquot used in the spectrophotometer cuvette. Lactic dehydrogenase was obtained from a commercial source; DPNH and the nucleotides were products of the Sigma Chemical Co., except as indicated. Routine assays were conducted by the addition of pyruvate kinase to appropriate cuvettes containing the following in a final volume of 3 ml: imidazole buffer, pH 7.5, 150 μmoles; PEP, 1 μmole; $MgCl_2$, 2 μmoles; DPNH to give an initial absorbance at 340 mμ of about 0.500; lactic dehydrogenase, 0.1 μl; nucleoside diphosphate, 2 μmoles.

With a sample of purified UDP*, an apparent lag period occurred before the oxidation of the DPNH (Fig. 1). Preincubation of this sample with pyruvate kinase but without PEP, followed by the addition of PEP to initiate the reaction, resulted in DPNH oxidation at a rate comparable to that observed when ADP was acting as a substrate. These results indicated that a reaction other than the direct phosphorylation of the UDP by the PEP was taking place. This reaction, I, catalyzed by nucleoside diphosphokinase, requires the presence of only trace amounts of adenine nucleotides as contaminants of the materials under study to generate sufficient ATP via reaction II so that the rate of reactions I and II increases in an autocatalytic fashion. Chromatographic analysis of commercial UDP indicated the presence of approximately 5 % of

Abbreviations: ADP, ATP, adenosine di- and tri-phosphate; CDP, CTP, cytidine di- and tri-phosphate; GDP, guanosine diphosphate; IDP, inosine diphosphate; UDP, uridine diphosphate; R, ribose; PEP, phosphoenolpyruvate; DPNH, reduced diphosphopyridine nucleotide.

* Obtained through the courtesy of Dr. RICHARD POTTER.

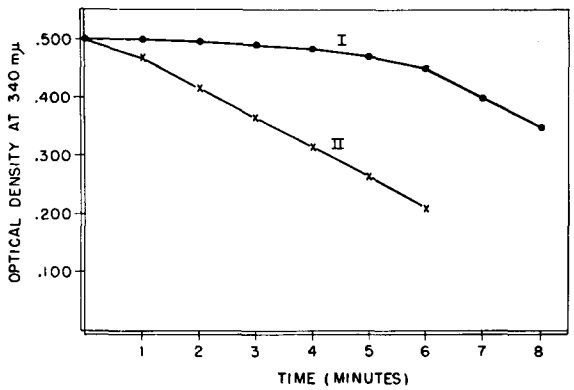

Fig. 1. Assay mixture contains per 3.0 ml: UDP, 2 μmoles; PEP, 1 μmole; $MgCl_2$, 1.5 μmoles; imidazole buffer, pH 7.5, 150 μmoles; lactic dehydrogenase, 5 μg; pyruvate kinase, 8 μg; DPNH to give the indicated absorbance. Curve I represents the course of the reaction initiated at zero time by the addition of the enzyme solutions; Curve II represents the course of the reaction when the complete system minus PEP was incubated for 5 min and the reaction started at zero time by the addition of PEP.

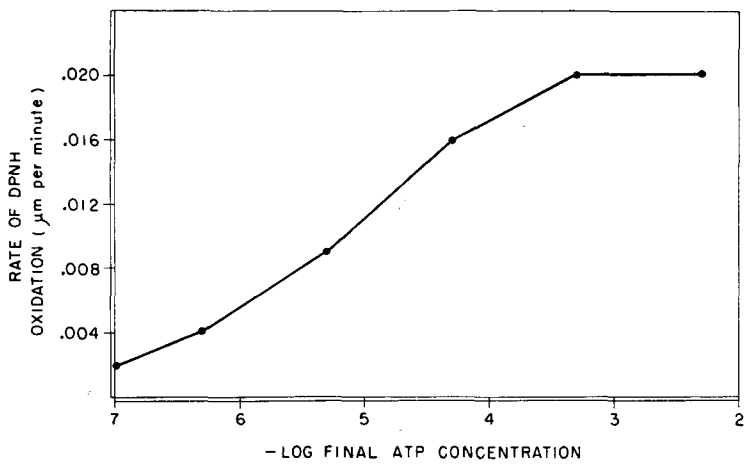

Fig. 2. Assay mixture as described for Fig. 1 except for the addition of the indicated amounts of ATP. Reaction started by the addition of the enzyme solutions.

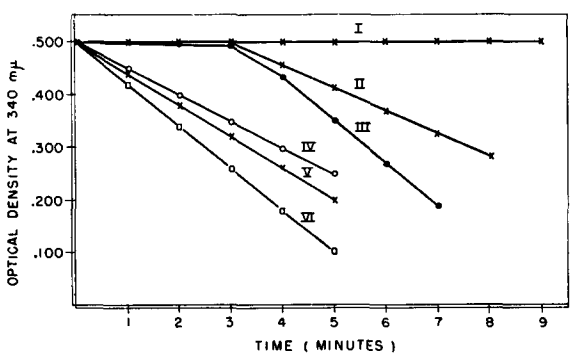

Fig. 3. Assay conditions as indicated in the text. Curve I, CDP; curve II, CDP to which 1.0 μmole ATP was added after 3-min incubation; curve III, UDP to which 1.0 μmole ATP was added after 3-min incubation; curve IV, GDP; curve V, IDP; curve VI, ADP.

adenine-containing nucleotides (presumably ATP) whereas similar examination of CDP and GDP failed to reveal any detectable contamination.

As indicated in Fig. 2, the addition of ATP to the reaction mixture results in the immediate oxidation of the DPNH at a rate which is dependent on the ATP concentration until saturation of the system is obtained. Apparently, at sub-optimal ATP concentrations, reaction I is rate limiting in the overall system whereas, at saturation levels of ATP, one of the other reactions may determine the rate.

Similar experiments were carried out with CDP, GDP and IDP, with ADP as control. The results are indicated in Fig. 3. As may be seen, CDP is completely inactive with pyruvate kinase even after extended incubation times. Both GDP and IDP function as a substrate for pyruvate kinase as has been previously reported and the addition of ATP to either of these substrates is not required for phosphorylation to take place. The corresponding nucleoside triphosphates can be demonstrated by paper chromatography[5] after incubation of ADP, GDP or IDP with pyruvate kinase and PEP alone. Similar triphosphate formation could be demonstrated after chromatography of incubation mixtures containing UDP, with or without added PEP; however, CTP formation from CDP required the presence of ATP. As previously indicated, analysis of the UDP preparation indicated sufficient contamination by adenine-containing nucleotides to account for the formation of UTP by the action of nucleoside diphosphokinase. UTP formation has also been reported to occur as a result of reaction between UDP and ADP[6].

Thus, the contamination of either lactic dehydrogenase or pyruvate kinase with nucleoside diphosphokinase may be utilized to assay either CDP or UDP by the addition of ATP to the system thereby coupling reaction I to reactions II and III. The extremely broad distribution of the latter enzyme makes it appear likely that other biochemical and physiological reactions which appear to utilize several nucleoside phosphates as substrates for a single enzyme (hexokinase, muscle contraction), may be specific for a single nucleotide.

The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan. This investigation was supported in part by a grant (No. A-512) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and a grant from the Michigan Chapter, Arthritis and Rheumatism Foundation.

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Received September 29th, 1958

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