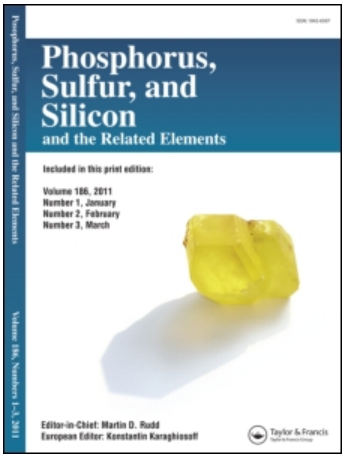

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CHARACTERIZATION OF THE ROLE OF INORGANIC PYROPHOSPHATASE IN REGULATING THE REACTION OF URIDINE 5'-TRIPHOSPHATE WITH GLUCOSE 1-MONOPHOSPHATE

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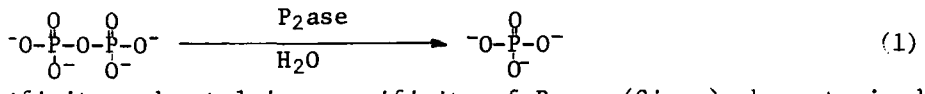
Abstract Inorganic pyrophosphatase (EC 3.6.1.1) drived the reaction of uridine 5'-triphosphate with glucose 1-monophosphate in the direction of uridine 5'-diphosphoglucose formation.

INTRODUCTION

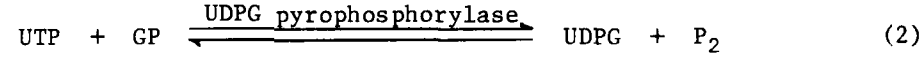
Inorganic pyrophosphatase (P_2ase, EC 3.6.1.1) isolated from baker's yeast, with the molecular weight of 64000, is known to catalyze the hydrolysis of inorganic pyrophosphate (diphosphate, P_2) to orthophosphate (P_1)[1-3]. In previous papers[4-8] we reported the substrate



(1)

specificity and metal ion specificity of P_2ase (Sigma) characterized by flow injection analysis (FIA) and high-performance liquid chromatography (HPLC). The P_2ase was concluded to be quite specific for P_2 in the presence of magnesium ion with the specific activity of as high as 580 U/mg at pH 7.2 and 25°C.

It has been assumed that P_2 may be involved as one of products in some biological reactions ($A+B \rightleftarrows C+P_2$) and its concentration level controllable by P_2ase may regulate such reactions in metabolic processes[2-3]. Three important examples are the formation of RNA and cyclic AMP from nucleoside triphosphates and the formation of uridine 5'-diphosphoglucose (UDPG) by the reaction of uridine 5'-triphosphate (UTP) with glucose 1-monophosphate (GP) in the presence of UDPG pyrophosphorylase (EC 2.7.7.9). This work in connection with eqn. 2 was undertaken to show an experimental evidence *in vitro* of supporting the above concept that has not always been accepted by all because of the lack of straightforward evidence based on kinetic experiments. P_2ase was expected to drive the equilibrated reaction in the direction of UDPG formation by lowering the concentration of P_2 that is chemically stable, but is rapidly hydrolyzed by P_2ase.



(2)

EXPERIMENTAL

Analytical Systems[4-8]

Analytical systems based on FIA and HPLC were designed which could be employed for the rapid analysis of phosphorus compounds in eqns. 1 and 2 with the detection of phosphorus. An FIA system employing a Mo(VI) reagent was useful for the selective determination of the product in the presence of the substrate in eqn. 1, permitting the automatic measurement of P_2ase activity. The rapid enzymatic hydrolysis with an apparent half-life of the order of 2 min was easily measured. An HPLC system using a Mo(V)-Mo(VI) reagent was employed for the simultaneous determination of various phosphorus compounds in eqns. 1 and 2. The Mo(V)-Mo(VI) reagent not only accelerated the hydrolysis of polymeric phosphates to P_1 in the reaction coil maintained at 140°C, but also reacted with the resultant P_1 to form a heteropoly blue complex with an absorption maximum at 822 nm. The reagent was easy to prepare and stable at least for 2 months[8].

In HPLC experiments with an anion-exchange separation column (4mm ID x 25cm, TSKgel SAX) the use of an eluent, 0.23M KCl + 1mM Na_2EDTA + 10mM NH_3 (pH10), was recommended for the isocratic elution of P_1, P_2 and tripolyphosphate. Two eluents for the gradient elution of phosphorus compounds in eqn. 2 were 0.12M KCl + 0.3mM Na_4EDTA + 0.7mM Na_3EDTA (Eluent A) and 0.29M KCl + 0.3mM Na_4EDTA + 0.7mM Na_3-EDTA (Eluent B).

RESULTS AND DISCUSSION

Substrate Specificity and Metal Ion Specificity[4-8]

Marked effects of alkaline earth metal ions and reaction temperature on the P_2ase activity were observed at pH 7.2. Only magnesium ion activated the enzymatic hydrolysis of P_2, and calcium and strontium ions inhibited its effect. The activity increased with increasing reaction temperature between 10-40°C. The Michaelis constant at 25°C and pH 7.2 was evaluated to be ca. 0.003mM. Substrate specificity was also investigated in the presence of various substrates, P_2, tripolyphosphate and ten oligophosphates. The P_2ase was found to be quite specific for P_2 in the presence of magnesium ion.

Careful preparation of purified water to be used as a solvent was needed to remove or deactivate pseudo-enzymatic substances in deionized water[8].

Effect of P_2ase on UDPG Formation

All HPLC profiles in Fig. 1 were obtained by gradient elution anion-exchange chromatography using a separation column (4mm ID x 25 cm, TSKgel SAX) and Eluents A and B (pH 9.8). Figure 1a shows an HPLC profile for a mixture of UTP and GP (each 0.2mM) incubated at pH 7.2 and 25°C in the presence of 2mM magnesium chloride. Aliquots of the mixture were injected at 1h intervals. Both UTP and GP as starting compounds were confirmed to be stable at least for 4h.

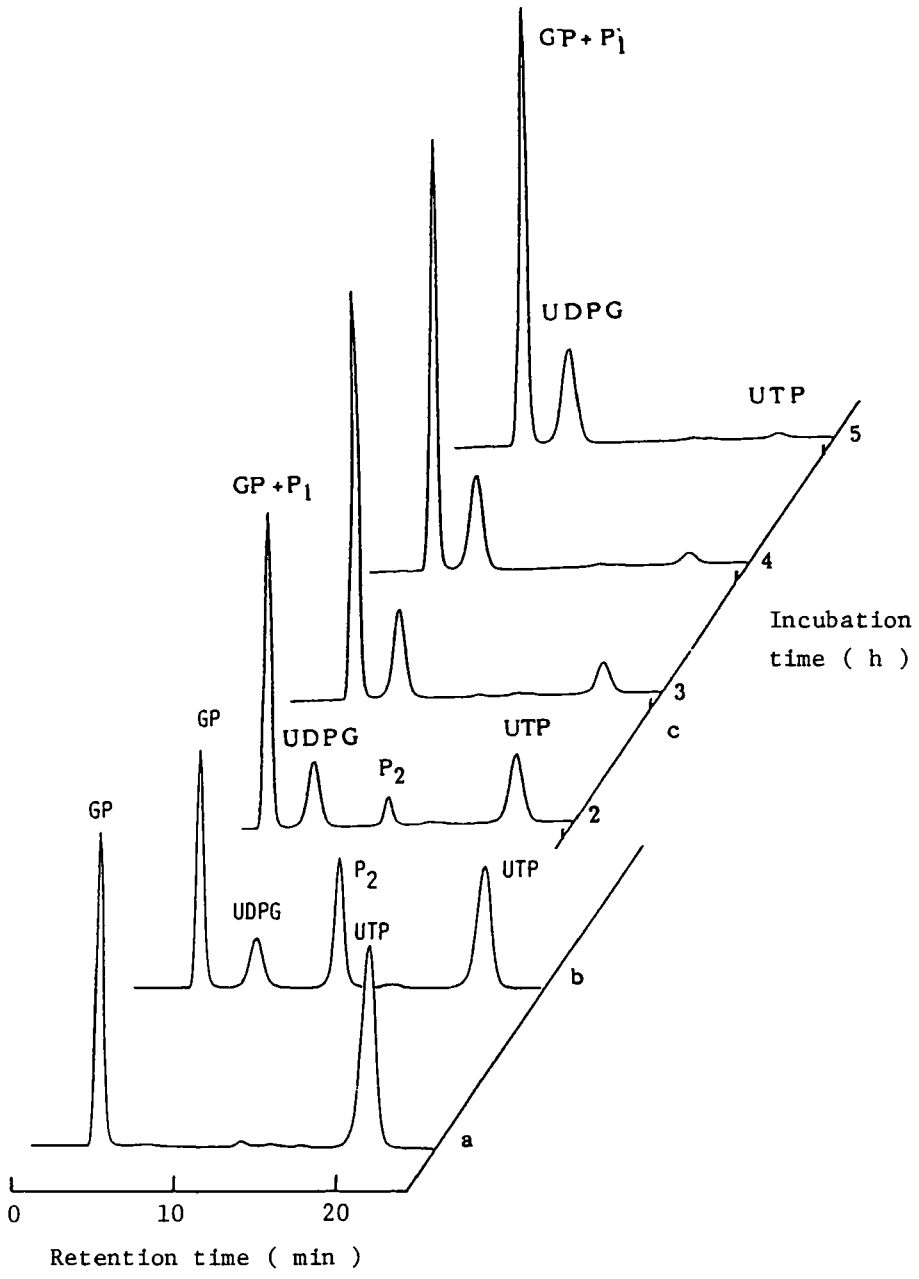


FIGURE 1. HPLC profiles for the reaction products between UTP and GP. (a) without enzyme, (b) with 2 x 10^{-7} M UDPG pyrophosphorylase, and (c) with subsequent addition of 1.5 x 10^{-10}M P_2ase to (b).

Figure 1b shows an HPLC profile obtained after 1h incubation under the same conditions as in Fig. 1a, except that 0.2μM UDPG pyrophosphorylase (Boehringer) was added. As expected the enzymatic reaction in eqn. 2 proceeded to produce UDPG and P_2. The profile was also unchanged at least 4h to indicate that four species are in equilibrium with each other. The approximate equilibrium constant was calculated to be 0.23, in close agreement with the value (0.22) in the literature[9]. The enzyme concentration in Fig. 1b was too high to monitor the time course of the formation of P_2 and UDPG within 1h. A separate experiment was done by lowering the concentration of UDPG pyrophosphorylase from 0.2μM (Fig. 1b) to 0.006μM. Both peaks of P_2 and UDPG increased gradually to attain an equilibrium after 4h, with the distribution of four species similar to that in Fig. 1b.

Marked change was observed when P_2ase (0.00015μM, Sigma) was added to the equilibrated solution in Fig. 1b. The peaks for P_2 and UTP decreased gradually with time, in contrast to the increase of the peak for UDPG. After 4h incubation almost all of P_2 and UTP disappeared as shown in Fig. 1c. As well as P_2 and UTP, GP was also expected to decrease with time. Unfortunately, its straightforward evidence was not available, because the GP peak overlapped with the P_1 peak that was produced by the hydrolysis of P_2. The decrease of GP could be indirectly estimated from the increase of UDPG having a common group.

The kinetic data obtained by HPLC with the detection system of phosphorus was satisfactory to visually indicate that P_2ase drived the reaction 2 in the direction of UDPG formation by decreasing the P_2 concentration and to support the concept that P_2ase may play an important role in regulating enzymatic reactions of the type; $A + B \rightleftharpoons C + P_2$.

REFERENCES

1. B.S. Cooperman, Methods Enzymol., 87, 526(1982).
2. M. Baltscheffsky, and P. Nyren, in New Comprehensive Biochemistry, edited by A. Neuberger, and L.L.M. Van Beenen (Elsevier, Amsterdam, 1984), Vol. 9, Chap. 6.
3. R.W. McGilvery, and G.W. Goldstein, Biochemistry; A Functional Approach (Saunders, Philadelphia, 1983).
4. N. Yoza, H. Hirano, M. Okamura, S. Ohashi, Y. Hirai, and K. Tomokuni, Chem. Lett., 1983, 1433.
5. N. Yoza, H. Hirano, Y. Baba, and S. Ohashi, J. Chromatogr., 325, 385(1985).
6. H. Hirano, Y. Baba, N. Yoza, and S. Ohashi, Anal. Chim. Acta, 179, 209(1986).
7. H. Hirano, Y. Baba, N. Yoza, and S. Ohashi, Chem. Lett., 1986, 633.
8. N. Yoza, Y. Sagara, H. Morioka, T. Handa, H. Hirano, Y. Baba and S. Ohashi, J. Flow Injection Anal., 3, 37(1986).
9. R.W. Guynn, D. Veloso, J.W.R. Lawson, and R.L. Veech, Biochem. J., 140, 369(1974).