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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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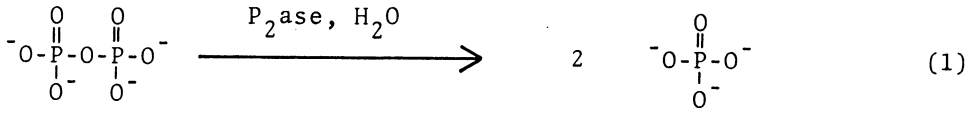
Inorganic pyrophosphatase (EC 3.6.1.1) catalyzing the hyrolysis of inorganic pyrophosphate drived the reaction of uridine 5'-triphosphate with glucose 1-monophosphate in the direction of uridine 5'-diphospho-glucose formation. The kinetic data *in vitro* supported the concept that inorganic pyrophosphate might be involved as one of products and its concentration level might regulate the above reaction in metabolic process.

Inorganic pyrophosphatase (P_2ase, EC 3.6.1.1) isolated from baker's yeast is known to catalyze the hydrolysis of inorganic pyrophosphate (diphosphate, P_2) to orthophosphate (P_1).[1-3]

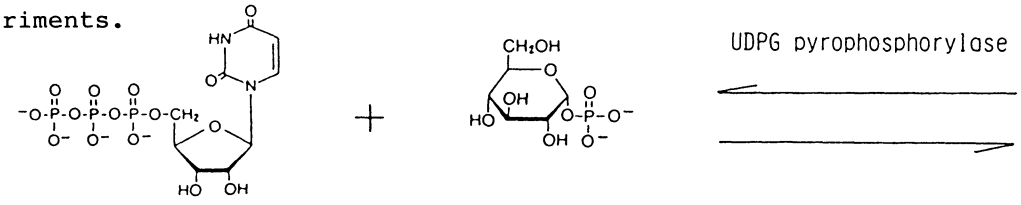


P_2 P_1 (1)

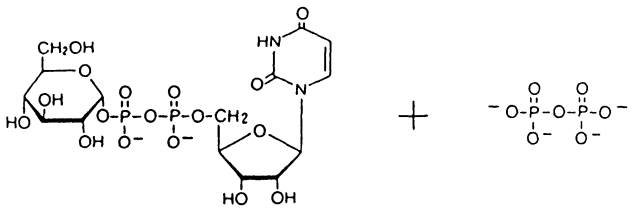
In previous papers [4-6] we reported the substrate specificity and metal ion specificity of P_2ase which were characterized by the analytical techniques of flow injection analysis (FIA) and high-performance liquid chromatography (HPLC). The P_2ase was concluded to be quite specific for P_2 in the presence of magnesium ion.

It has been assumed[2,3] that P_2 may be involved as one of products in some biological reactions and its concentration level controllable by P_2ase may regulate such reactions in metabolic process. 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 Eq. 2 was undertaken to show an experimental evidence *in vitro* of supporting the above assumption that has not always been accepted by all because of the absence of detectable amount of P_2 in biological systems and the lack of straightforward evidence based on kinetic experiments.



Uridine 5'-triphosphate Glucose 1-monophosphate



Uridine 5'-diphosphoglucose Pyrophosphate (2)

Since the product P_2 in Eq. 2 is chemically stable with a half life of about three years in a neutral medium at 30 °C, it has to be hydrolyzed enzymatically according to Eq.1 to drive the equilibrated reaction (Eq. 2) to the right. The marked effect of P_2ase on the reaction in Eq.2 was shown by an HPLC technique that enabled the simultaneous determination of four species in Eq.2.

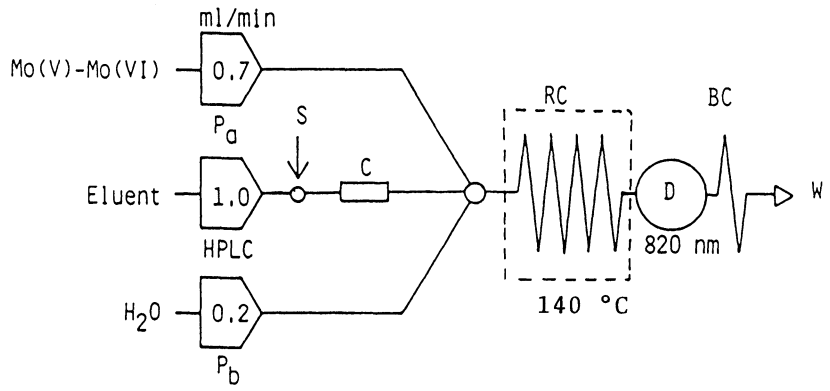


Fig. 1. High-performance liquid chromatographic system for inorganic and organic phosphorus compounds. C, separation column (4 mm ID x 5 cm); RC, reaction coil (0.5 mm ID x 20 m);D, spectrophotometer; BC, back-pressure coil; S, sample injector; W, waste.

The HPLC system in Fig. 1 is similar to that used in the previous paper.[6] This system is based on the anion exchange separation using a TSK-gel SAX (10 μm) and the post column detection of phosphorus using a molybdenum(V)-molybdenum(VI) reagent. The reagent reacted with orthophosphate to form a heteropolyblue complex which could be detected at its absorption maximum, 820-830 nm. The hydrolysis of inorganic and organic phosphorus compounds to orthophosphate and the color reaction proceeded simultaneously in the reaction coil (RC) maintaied at 140 °C.

HPLC profiles in Fig. 2 were obtained by gradient elution anion exchange chromatography using 0.12 - 0.29 M potassium chloride (pH 9.8, M = mol dm^{-3}) as eluents. Detailed procedures will be described in a subsequent paper of this series. Figure 2(a) shows a profile for a mixture of UTP and GP (each 2 x 10^{-4} M) incubated at pH 7.2 and 25 °C for 1 h in the presence of 2 x 10^{-3} M magnesium chloride. The profile was unchanged at least for 4 h. Figure 2(b) is a profile obtained under the same conditions as in Fig. 2(a), except that 2 x 10^{-7} M UDPG pyrophosphorylase (Boehringer) was added. As expected the enzymatic reaction in Eq.2 proceeded to produce UDPG and P_2. This profile was also unchanged at least for 4 h to indicate that four species are in equilibrium with each other. The approximate value of the equilibrium constant was estimated to be 0.23.

Marked change in an HPLC profile was observed when P_2ase (1.5 x 10^{-10} M ,Sigma) was added to the equilibrated solution in Fig. 2(b). The peaks for P_2 and UTP decreased gradually with time, in contrast to the increase of the UDPG peak. After 4 h almost all of P_2 and UTP disappeared as shown in Fig. 2(c). As well as P_2 and UTP, GP was expected to decrease. Unfortunately, its straightforward evidence was not available from Fig. 2(c), because the GP peak overlapped with the P_1 peak that was produced by the hydrolysis of P_2.

There are general ways of changing the rate of an enzymatic reaction;[3] the alteration of the catalytic activity of an enzyme through the use of effectors and the alteration of product concentrations. The control by P_2ase of the rate of UDPG formation in Eq.2 is based on the alteration of P_2 concentration. The kinetic data obtained by high-performance liquid chromatography with the detection system of phosphorus were satisfactory to support the concept that P_2ase may play an important role in regulating enzymatic reactions that include inorganic pyrophosphate as one of products. Detailed results will be dicussed elsewhere.

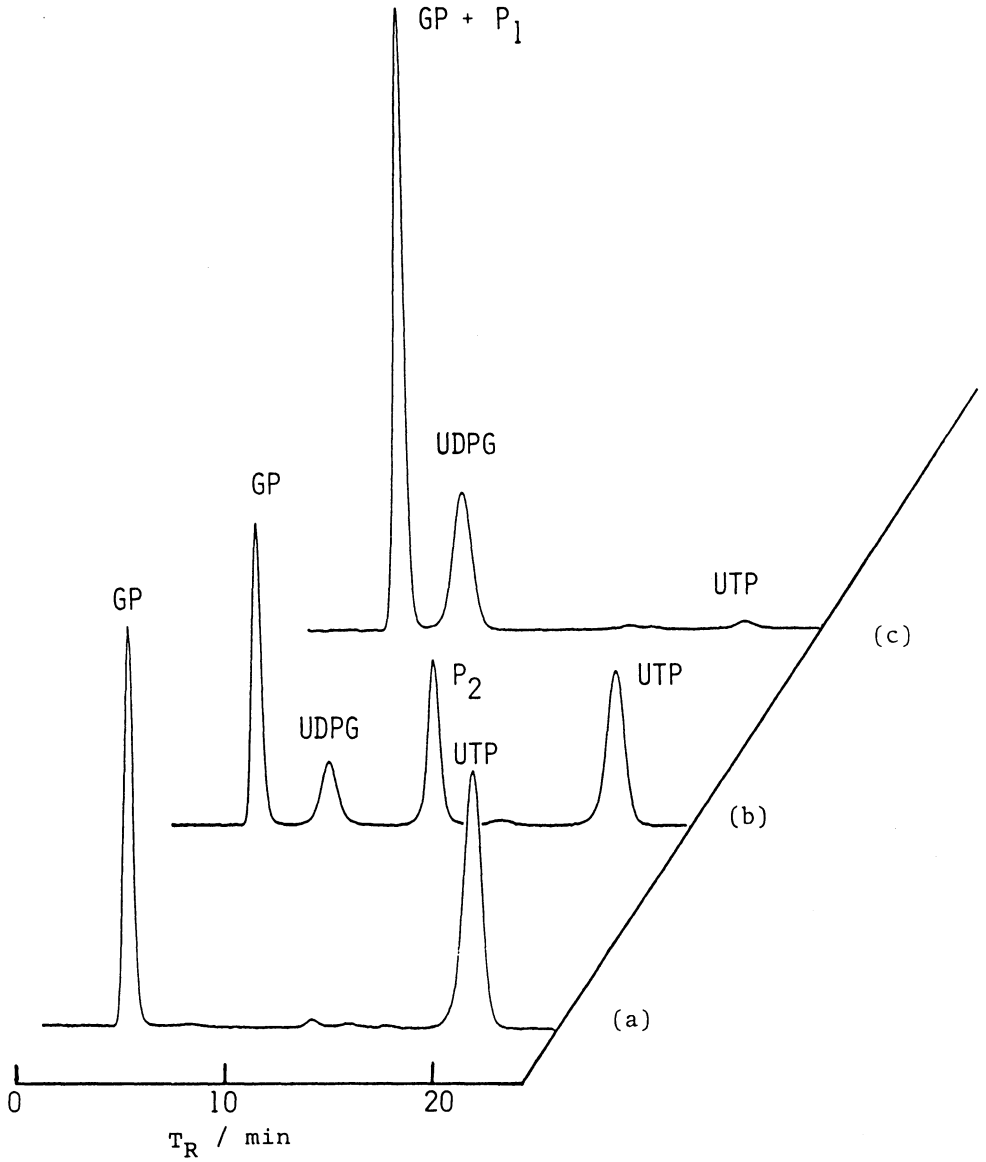


Fig. 2. HPLC profiles for the reaction products between UTP and GP.
(a) without enzyme, (b) with UDPG pyrophosphorylase, and
(c) with UDPG pyrophosphorylase and P_2ase. T_R is retention time.

References

1) B. S. Cooperman, Methods Enzymol., 87, 526(1982).

2) R. W. McGilvery and G. W. Goldstein, " Biochemistry; A Functional Approach," Saunders, Philadelphia (1983).

3) L. Stryer, " Biochemistry," Freeman, San Francisco (1975).

5) N. Yoza, H. Hirano, M. Okamura, S. Ohashi, Y. Hirai, and K. Tomokuni, Chem. Lett., 1983, 1433.

6) N. Yoza, H. Hirano, Y. Baba, and S. Ohashi, J. Chromatogr., 325, 385(1985).

7) H. Hirano, Y. Baba, N. Yoza, and S. Ohashi, Anal. Chim. Acta, in press.

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