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PHOSPHORYLATION OF INOSINE WITH CYCLO-TRIPHOSPHATE

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Phosphorylation of inosine with cyclo-triphosphate(P_{3m}) in aqueous solution was studied by means of anion-exchange chromatography and high performance liquid chromatography. P_{3m} reacted with inosine at 70°C and room temperature and at pH 12 to form inosine 2'- and 3'-monophosphates. The yields of 2'- and 3'-monophosphates were about 33 and 26%, respectively.

At present, as phosphorylating agents for nucleosides and related organic compounds are known phosphorus oxychloride [1 — 3] and various organic phosphorus compounds.[1, 4 — 6] However, the phosphorylation of nucleosides with such reagents is generally complicated. Also, phosphorylation of nucleosides with inorganic phosphates has been reported by Schwartz,[7, 8] Saffhill,[9] and Schoffstall.[10] Authors, studying phosphorylation reactions of various organic compounds with condensed phosphates,[11] especially cyclo-phosphates, found that inosine can be easily phosphorylated with cyclo-triphosphate. As phosphates of inosine, are generally well known inosine 5'-(5'-IMP), 3'-(3'-IMP), 3',5'-cyclic monophosphate(3',5'-cIMP), 5'-diphosphate (5'-IDP), and 5'-triphosphate(5'-ITP) and especially 5'-IMP, which shows flavoring properties, is used in daily life in great quantities. But, as to other inosine phosphates(inosinic acids), methods of their preparation and their properties seem not to be satisfactorily studied.

In the present study, an 0.5 mol dm^{-3} inosine solution was mixed with a 0.5 mol dm^{-3} sodium cyclo-triphosphate(P_{3m}) solution, the pH of the mixed solution was adjusted to 12 with a 6 mol dm^{-3} sodium hydroxide solution, and allowed to stand at room temperature and at 70°C. As the pH of the solution gradually lowered with the progress of the reaction, the sodium hydroxide solution was added to the solution to keep its pH at 12.

The identification and the determination of reaction products were carried out by means of anion-exchange chromatography and high performance liquid chromatography (HPLC). For the anion-exchange chromatography, a column of 64 X 1.5 cm I. D., packed with an anion-exchange resin Dowex 1-X2(formate form) of 200 — 400 mesh, was used. As an eluent was used a mixed solution of 0.1 mol dm^{-3} sodium formate and 1.0 mol dm^{-3} formic acid. The pH of the eluent was about 2.5. One cm^3 of the sample was injected into a column and the eluate was fractionated in each 10-g portion with a fraction collector. Inosine and inosinic acids were determined by means of spectrophotometry at 250 nm. HPLC was carried out by use of Shimadzu LC-3A, under the following conditions: packing material H_2N-Silica, column size 250 X 4.6 mm I. D., pressure 70 kg/cm^2, column temperature 40 °C, eluent 0.015 mol dm^{-3} ammonium dihydrogen orthophosphate, flow rate 1.0 cm^3/min.

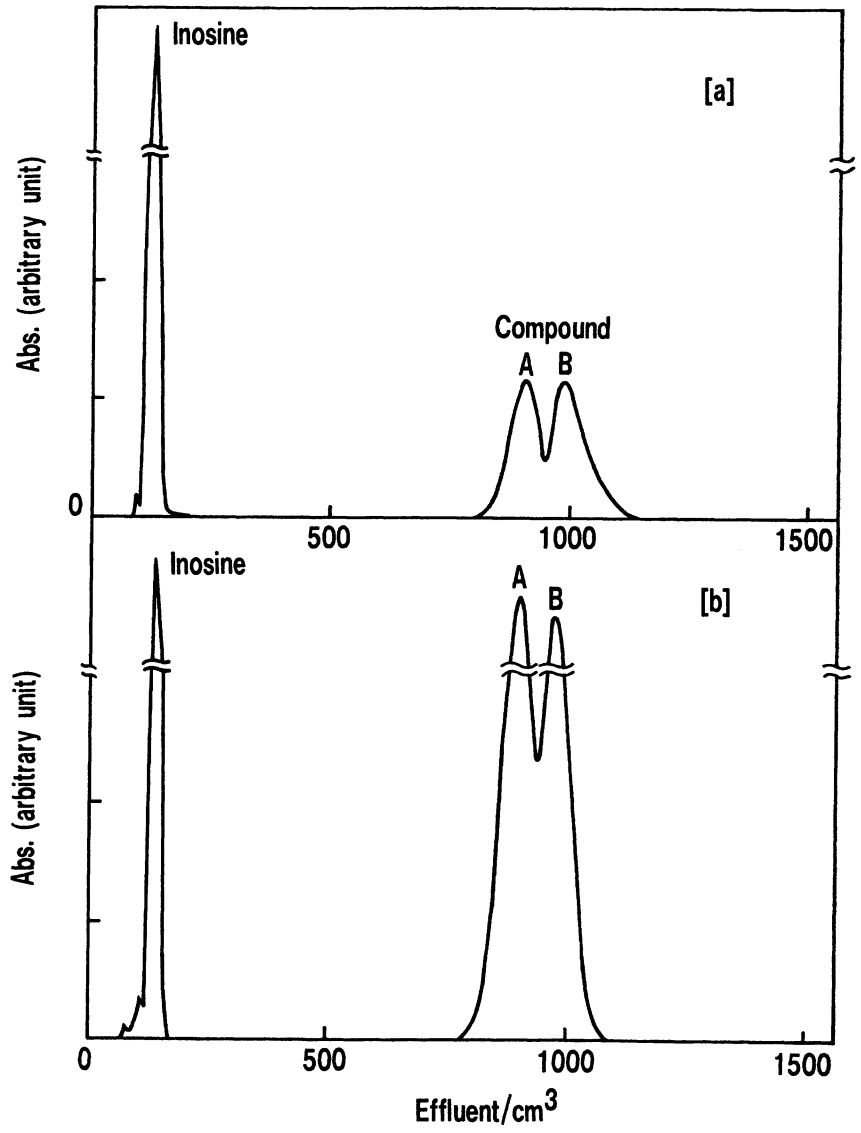

Fig. 1 Elution patterns for the reaction products of P_{3m} with inosine at 70°C and pH12
Absorbance was measured at 250 nm
[a] : After 1 day, [b] : After 7 days

Anion-exchange chromatograms, obtained at 1 and 7 d after the reaction of inosine with P_{3m} at 70°C, are shown in Fig. 1. As can be seen, two elution peaks of unknown compounds (hereafter denoted as compounds A and B) are observed at about 900 and 980 cm^3. The elution peak of inosine is also observed at 140 cm^3. It is proved that the amounts of compounds A and B increase with the reaction time.

Figure 2 shows the results of absorbance

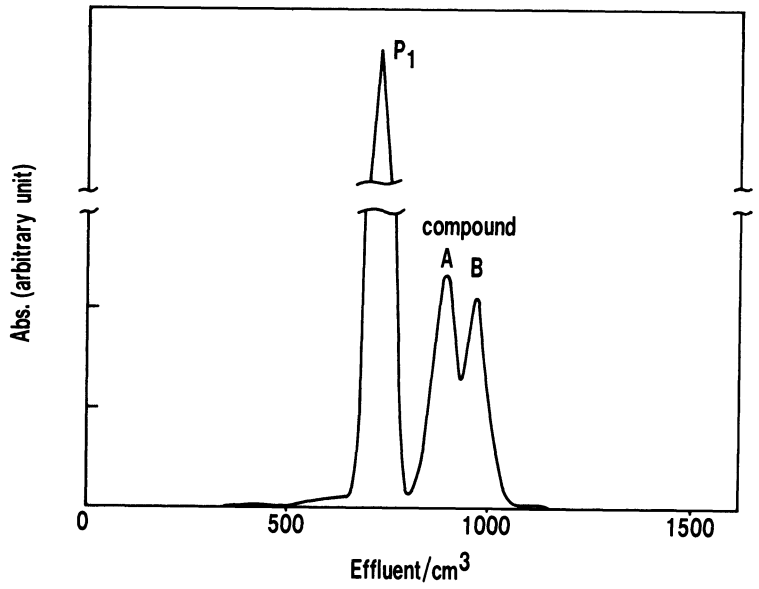

Fig. 2 Elution pattern for the reaction products of P_{3m} with inosine at 70°C and pH12
Absorbance was measured at 830 nm
After 43 days

measurements for phosphorus in the effluent, carried out by the orthophosphoric heteropoly-blue method. The absorbance was measured at 830 nm. In Fig. 2, after the elution peak of orthophosphate(P_1) at 730 cm^3, two peaks of compounds A and B are observed. Therefore, based on the results shown in Figs. 1 and 2, it is suggested that compounds A and B may be inosinic acids.

Based on anion-exchange chromatograms as well as on high performance liquid chromatograms of standard reagents of 5'-IMP, 3'-IMP, 3',5'-cIMP, 5'-IDP, and 5'-ITP, compound B was proved to be 3'-IMP. Compound A could not perfectly be identified yet, but some results obtained suggest that compound A may be 2'-IMP.

As can be seen from Fig. 3, yields of 2'-IMP and 3'-IMP, formed by the reaction of inosine with P_{3m} at pH 12 and at 70°C, increase rapidly up to 7 d, but after that time, they remain almost constant, about 33% for 2'-IMP and about 26% for 3'-IMP.

On the other hand, the phosphorylation reaction of inosine with P_{3m} at room temperature proceeded somewhat slowly compared with that at 70°C, and 2'-IMP and 3'-IMP were 22.9 and 15.3% even after 50 d, respectively. The phosphorylation of inosine with P_{3m}became more difficult with the lowering of pH, and did not proceed at all in an acidic medium.

Hypoxanthine, the purine base of inosine, was not phosphorylated at all with P_{3m} under above-described experimental conditions.

The phosphorylation of inosine with short-chain phosphates, such as ortho, pyro, and triphosphate, did not also proceed.

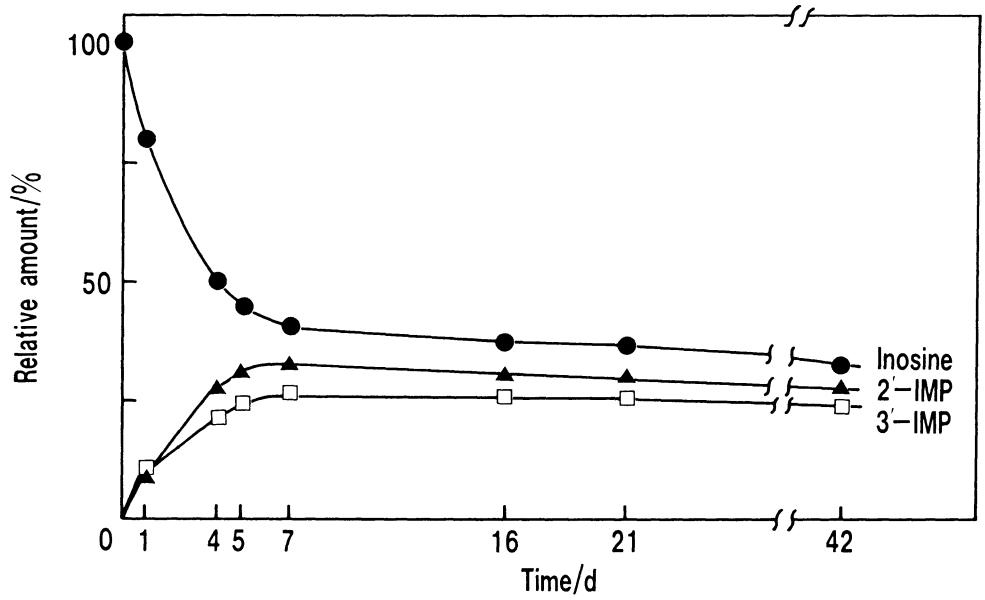

Fig. 3 Change of the amounts of 2'-IMP, 3'-IMP, and inosine in the reaction of P_{3m} with inosine
P_{3m} : Inosine=1 : 1, pH12, 70°C

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