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Phosphonylation of Glucose 1-Monophosphate with Diphosphonate

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Glucose 1-monophosphate was "phosphonylated" by diphosphonate in an aqueous solution under the mild conditions (60-70 °C, pH 6). Diphosphonate phosphonylated a phosphate group on glucose 1-monophosphate selectively. The yield of a phosphonylated product was 61%. The chemical structure of a phosphonylated product was discussed on its ^{31}P-NMR spectrum and high performance liquid chromatogram.

Disodium diphosphonate (P^{III}-O-P^{III}), which is an anhydride of phosphonate with phosphorus of oxidation number of 3, reacts with several nucleophiles.[1-5] In this reaction, the "phosphonyl group" (H-$\overset{O}{\overset{\|}{P}}$-$O^-$) of P^{III}-O-P^{III} is transferred to the nucleophile (X) to form a "phosphonylated product" (X-$\overset{O}{\overset{\|}{P}}$-H O^-) and phosphonate (P^{III}) as shown in Eq. 1.

$$\underset{P^{III}\text{-O-}P^{III}}{H\text{-}\overset{O}{\overset{\|}{P}}(O^-)\text{-O-}\overset{O}{\overset{\|}{P}}(O^-)\text{-}H} + X \longrightarrow X\text{-}\overset{O}{\overset{\|}{P}}(O^-)\text{-}H + \underset{P^{III}}{{}^-O\text{-}\overset{O}{\overset{\|}{P}}(O^-)\text{-}H} \qquad (1)$$

This type of reaction can be termed "phosphonyl transfer reaction" or "phosphonylation" because the reaction is similar to phosphoryl transfer reaction or phosphorylation of biological importance.[6]

P^{III}-O-P^{III} phosphonylated a phosphate group on adenosine 5'-monophosphate (AMP) to form a new compound with a POP bond, of which structure is similar to adenosine 5'-diphosphate (ADP).[1] The reaction proceeds under mild conditions without any enzymatic and chemical catalyses.[1] Phosphonylation with P^{III}-O-P^{III} is useful for the synthesis of a new compound with a POP bond, however, phosphonylation of biomolecules has not been well characterized. The purpose of this paper is to characterize a phosphonylated product by use of high-performance liquid chromatography (HPLC) and ^{31}P-NMR spectroscopy when disodium glucose 1-monophosphate (Glc1P) was used as X in Eq. 1. Phosphate and/or hydroxyl groups on Glc1P were expected to be phosphonylated by diphosphonate.

The reaction mixture (P^{III}-O-P^{III}; 1.0 mol dm^{-3}, Glc1P; 0.1 mol dm^{-3}) was incubated at 60 °C in the pH range of 6 (at 0 min) to 5.5 (at 100 min) without any buffer solution. Aliquots (0.1 ml) of the mixed solution were withdrawn at every 20 min and diluted with 100 ml distilled water. An aliquot (0.1 ml) of the diluted solution was introduced into an HPLC system designed for the rapid determination of both phosphate groups and phosphonate groups.[7-9] In this study only phosphate groups were detected in order to determine Glc1P and a phosphonylated product simultaneously.

The HPLC profile at time 0 in Fig. 1 shows the peak of only Glc1P. The peak area of a phosphonylated product (peak A in Fig. 1) increased with time at the expense of Glc1P. The phosphonylated product was present in maximum yield after 100 min, and then gradually disappeared. The appearance of only one peak for the phosphonylated product suggests the preferential phosphonylation of one of a hydroxyl group or a phosphate group on Glc1P. The yield of a phosphonylated product at 100 min was calculated to be 61% from the ratio of peak area of (A) to sum of areas for two peaks.

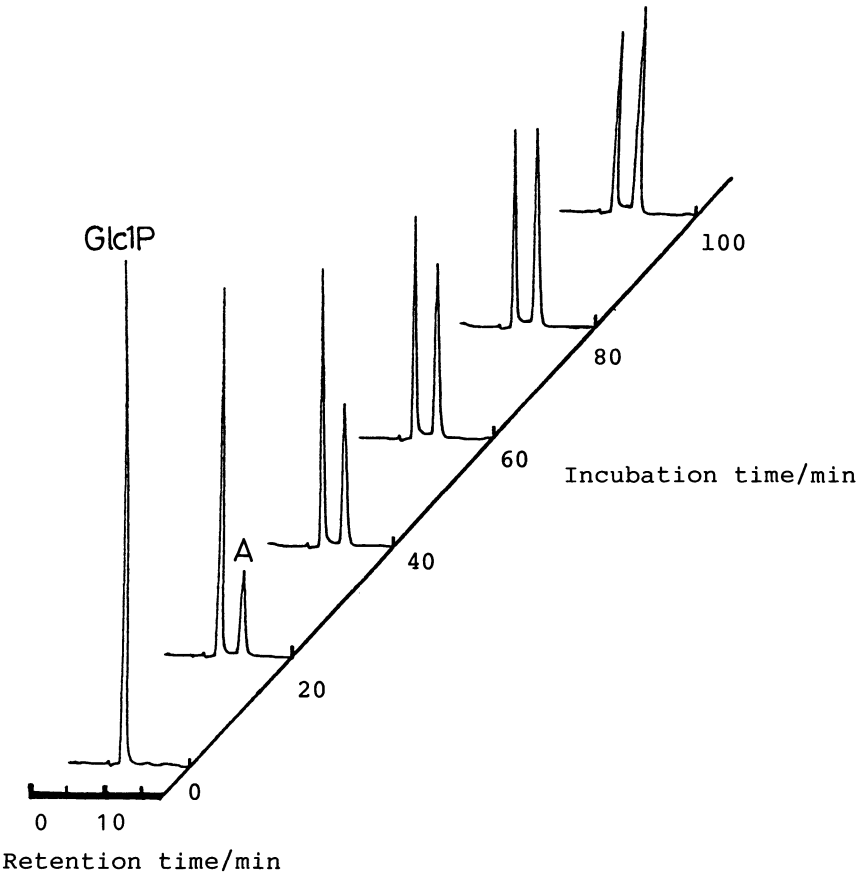


Fig. 1. Kinetic HPLC profile for phosphonylation of Glc1P (0.1 mol dm^{-3}) with diphosphonate (1.0 mol dm^{-3}) at 60 °C and pH 6. Column: 250x4.6 mm I.D., porous anion exchanger (TSKgel SAX, 10 μm). Column temperature: 40 °C. Eluent: 0.20 mol dm^{-3} potassium chloride and 0.1%(W/V) Na_4EDTA (pH 10). Flow rate : 1.0 ml/min.

In order to determine which one of the hydroxyl group or phosphate group on Glc1P was phosphonylated ^{31}P-NMR spectrum of the reaction mixture (P^{III}-O-P^{III}; 0.5 mol dm^{-3}, Glc1P; 0.1 mol dm^{-3}) was obtained at room temperature on a Varian Associates XL-300 spectrometer operated at 121 MHz. Chemical shifts were measured in comparison with a 85% phosphoric acid external standard, with positive shifts being down field. Proton-decoupled and proton-coupled spectra were shown in Figs. 2 and 3.

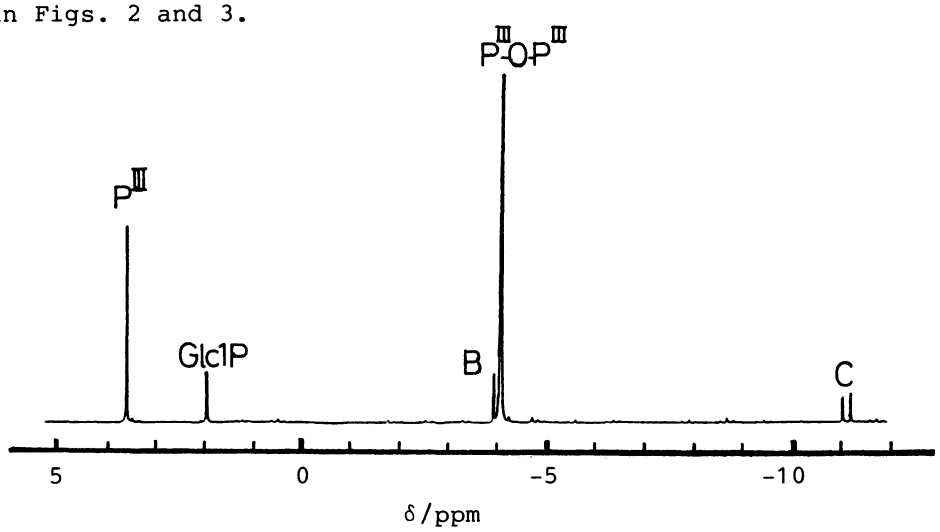


Fig. 2. Proton-decoupled ^{31}P-NMR spectrum at 121 MHz for phosphonylation of Glc1P (0.1 mol dm^{-3}) with diphosphonate (0.5 mol dm^{-3}) at 70 °C and pH 6. Incubation time: 100 min.

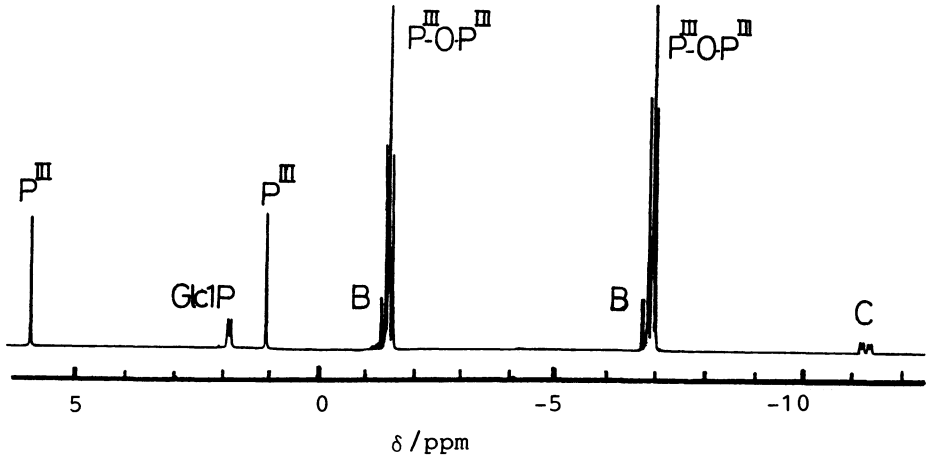
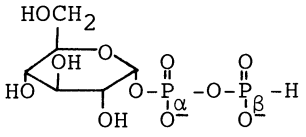


Fig. 3. Proton-coupled ^{31}P-NMR spectrum for phosphonylation of Glc1P with diphosphonate. Experimental conditions as in Fig. 2.

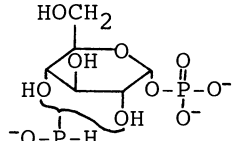
A proton-decoupled spectrum (Fig. 2) shows three singlets and two doublets. These singlets are assigned to P^{III} (3.57 ppm), Glc1P (1.90 ppm) and P^{III}-O-P^{III} (-4.26 ppm) on the basis of the chemical shifts for reference samples. Two unknown doublets (B and C, -4.19 and -11.4 ppm) are expected to be the signals for a phosphonylated product. They have the same J-value of 20.0 Hz. One of the signals for the doublet (B) is overlapped with the signal of P^{III}-O-P^{III}.

The new POP bond is likely to be formed by phosphonylation of Glc1P because

the magnitudes of J-values for both doublets are approximately equal to the geminal coupling constant ($^2J_{POP}$) in adenosine 5'-diphosphate (21.7 Hz).[10] The POP bond formation suggests that phosphate group on Glc1P is phosphonylated to form the phosphonylated product (**1**) as shown below. No positive evidence is obtained for the POC bond formation (**2**) by phosphonylation of OH groups on Glc1P, because OH groups are not reactive in a neutral aqueous medium.[11]



(**1**)



(**2**)

Both doublets (B and C) are further split into double-doublets shown in a proton-coupled spectrum (Fig. 3). The double-doublet (B) is assigned to the phosphorus atom (P_β of **1**) of phosphonyl group ($-\overset{O}{\overset{\|}{P}}(O^-)-H$) transferred from P^{III}-O-P^{III} to the phosphate group on Glc1P because the double-doublet (B) shows a splitting of 665.5 Hz for hydrogen attached directly to phosphorus atom.[12] The magnitude of J-values (7.26 Hz) for the double-doublet (C) is similar to the vicinal coupling constants ($^3J_{POCH}$) observed in Glc1P (6.90 Hz) and AMP (4.9 Hz).[13] Therefore, the double-doublet (C) is assigned to the signal of P_α of **1**. The analysis of the ^{31}P-NMR spectra is confirmed to be the formation of the phosphonylated product **1**.

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References

1) Y. Baba, Y. Yamamoto, N. Yoza, and S. Ohashi, Nucleic Acids Res. Symp. Ser., No. 17, 69(1986).
2) B. Blaser and K.H. Worms, Z. Anorg. Allg. Chem., 301, 18(1959).
3) R.E. Mesmer and R.L. Carroll, J. Am. Chem. Soc., 88, 1381(1966).
4) N. Yoza, M. Tokuda, T. Inoue, and S. Ohashi, Inorg. Nucl. Chem. Lett., 9, 385(1973).
5) N. Yoza, K. Ishibashi, and S. Ohashi, J. Chromatogr., 134, 497(1977).
6) J.R. Knowles, Ann. Rev. Biochem, 4, 877(1980).
7) Y. Baba, N. Yoza, and S. Ohashi, J. Chromatogr., 295, 153(1984).
8) Y. Baba, N. Yoza, and S. Ohashi, J. Chromatogr., 348, 27(1985).
9) Y. Baba, Y. Yamamoto, N. Yoza, and S. Ohashi, Bunseki Kagaku, 34, 692(1985).
10) H.J. Vogel and W.A. Bridger, Biochemistry, 21, 394(1982).
11) M. Tsuhako, M. Fujimoto, S. Ohashi, H. Nariai, and I. Motooka, Bull. Chem. Soc. Jpn., 57, 3274(1984).
12) D.G. Gorenstein, "Phosphorus-31 NMR Principles and Applications," Academic Press, Orlando (1984), Part 1-2, p. 39.
13) P.J. Cozzone and O. Jardetzky, Biochemistry, 15, 4860(1976).

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