Selective Synthesis of β-D-Glucopyranosyl 1-Triphosphate by Reaction of D-Glucopyranose with Inorganic cyclo-Triphosphate

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Received October 23, 1997; accepted December 5, 1997

The stereoselective phosphorylation of D-glucopyranose has been achieved using inorganic sodium cyclo-triphosphate hexahydrate (P_{3m}), $Na_3P_3O_9 \cdot 6H_2O$. The reaction product has been confirmed to be β-D-glucopyranosyl 1-triphosphate by multinuclear NMR and HPLC, and its maximum yield was about 47%.

Key words phosphorylation; multinuclear NMR; β-D-glucopyranosyl 1-triphosphate; cyclo-triphosphate hexahydrate; HPLC

Phosphate esters of monosaccharides are biologically important compounds. As is well known, α-D-glucopyranosyl 1-phosphate (glucose 1-phosphate) and α-D-glucopyranosyl 6-phosphate (glucose 6-phosphate) are intermediates in the glycolytic pathway, while β-D-sugar phosphates are key intermediates for the synthesis of sugar nucleotides.[1)]

Feldmann demonstrated that inorganic cyclo-triphosphate (P_{3m}), which is a simple inorganic condensed phosphate having a six-membered ring, could phosphorylate organic compounds such as alcohols and amino acids.[2)] In addition, the present authors have made a detailed study of the phosphorylation of alkylamines,[3)] sugars,[4)] cellulose,[5)] amino acids,[6)] phenols,[7)] nucleosides,[8)] and nucleotides[9)] with P_{3m}.

In the reaction of D-glucopyranose with P_{3m}, the results of HPLC and ^{31}P-NMR spectra indicated that the main product was the triphosphate derivative of D-glucopyranose and its maximum yield was about 47%.[4)] However, it was impossible to specify the position of the hydroxyl group phosphorylated in the D-glucopyranose molecule by ^{31}P-NMR and HPLC. In this paper it will be shown by multinuclear NMR that D-glucopyranose reacts with P_{3m} to form β-D-glucopyranosyl 1-triphosphate stereoselectively.

Results and Discussion

Figure 1(A) shows the ^{13}C-NMR spectrum of the reaction mixture of D-glucopyranose (2.5 mol·dm^{-3}) and P_{3m} (0.5 mol·dm^{-3}) incubated at room temperature and pH 12 for 1 d. In addition to the large signals of α- and β-D-glucopyranose, six small peaks (100.2, 79.0, 77.7, 76.1, 72.0, and 63.3 ppm) due to the main product were observed. In order to distinguish the signals of the main product from those of the reactant, the reactions were performed at different molar ratios (3 : 1 and 1 : 1) as shown in Figs. 1(B) and 1(C). The ratio of the peak height of phosphorylated product to D-glucopyranose decreased with the increase in the molar ratio of D-glucopyranose to P_{3m}. As the chemical shift values of α- and β-D-glucopyranose depend on pH, ^{13}C-NMR measurement of α- and β-D-glucopyranose was performed at pH 12 to assign the peaks of the reactant correctly. From the results, the peaks marked with an asterisk in Fig. 1 could be assigned to the phosphorylated product and the other peaks to α- and β-D-glucopyranose. After a long time the main product gradually decomposed to the monophosphate derivative of D-glucopyranose and pyrophosphate ($P_2O_7^{4-}$). It was difficult to distinguish between tri- and monophosphate derivatives of D-glucopyranose from their ^{13}C-NMR spectra. Although ^{31}P-NMR and HPLC measurements showed that the reaction product of D-glu-

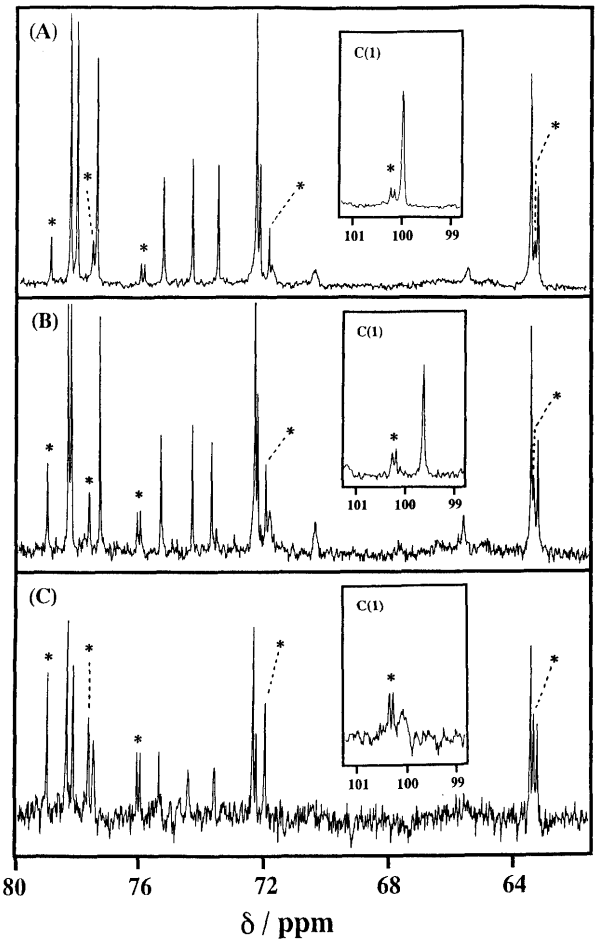

Fig. 1. The Broad Band Decoupled 75 MHz ^{13}C-NMR Spectra of the Reaction Solution of D-Glucopyranose and P_{3m} under the Following Conditions: (A) D-Glucopyranose (2.5 mol·dm^{-3}) and P_{3m} (0.5 mol·dm^{-3}) at pH 12 for 1 d, (B) D-Glucopyranose (1.5 mol·dm^{-3}) and P_{3m} (0.5 mol·dm^{-3}) at pH 12 for 2 d, (C) D-Glucopyranose (0.5 mol·dm^{-3}) and P_{3m} (0.5 mol·dm^{-3}) at pH 12 for 3 d

* shows the signals due to the main product, and the other peaks are due to α- and β-D-glucopyranose.

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Table 1. ^{13}C-NMR Chemical Shifts and Coupling Constants of α-,β-D-Glucopyranose and D-Glucopyranosyl 1-Phosphates

Compound	pH	Chemical shift (ppm)						Coupling constant (Hz)	
		C(1)	C(5)	C(3)	C(2)	C(4)	C(6)	$J_{PC(1)}$	$J_{PC(2)}$
β-D-Glucopyranosyl 1-triphosphate	12	100.2	79.0	77.7	76.1	72.0	63.3	5.6	8.2
α-D-Glucopyranose	12	95.5	73.6	75.5	74.6	72.3	63.2		
β-D-Glucopyranose	12	100.3	78.5	78.1	77.7	72.5	63.5		
α-D-Glucopyranosyl 1-phosphate	12	96.1	74.5	75.6	74.7	72.3	62.2	5.3	5.3
β-D-Glucopyranosyl 1-phosphate	12	99.7	79.0	78.3	77.1	72.7	63.9	4.7	6.2

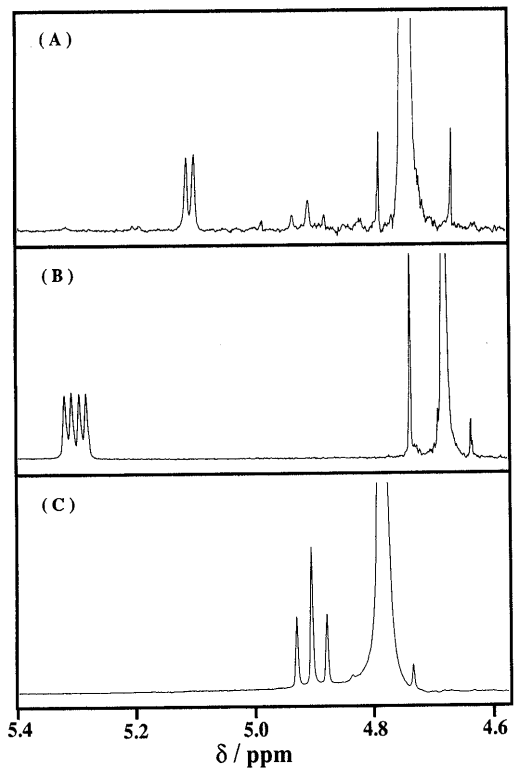


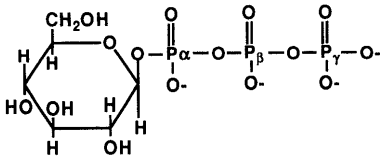
Fig. 2. The 300 MHz ^{1}H-NMR Spectra in the Region of H(1) of (A) the Main Product Formed by Reaction of D-Glucopyranose (2.5 mol·dm^{-3}) with P_{3m} (0.5 mol·dm^{-3}) at pH 12 for 1 d, (B) α-D-Glucopyranosyl 1-Monophosphate (Disodium Salt, 0.53 M in D_2O), (C) β-D-Glucopyranosyl 1-Monophosphate (Disodium Salt, 0.082 M in D_2O)

The large signals at 4.7 ppm (A, B, C) are due to residual H in water.

copyranose and P_{3m} is D-glucopyranose triphosphate,[4] the position of the phosphorylated hydroxyl group in D-glucopyranose could not be confirmed by ^{31}P-NMR and HPLC.

The ^{13}C-NMR measurements at different magnetic fields (4.70 and 7.05 T) suggested that the doublets at 100.2 and 76.1 ppm are due to coupling with P. Table 1 shows the ^{13}C chemical shift values of β-D-glucopyranosyl triphosphate together with the authentic samples (α- and β-D-glucopyranosyl 1-phosphates, and α- and β-D-glucopyranoses). Baker *et al.*[10] thoroughly investigated the ^{13}C- and ^{1}H-NMR spectra of α- and β-aldohexose 1-monophosphates, and α- and β-D-aldohexose cyclic 1, 2-monophosphates. The ^{13}C-NMR signals shift less than 1 ppm due to phosphorylation of the hydroxyl group at C(1) of aldohexoses, and coupling between P and C appears at C(1) and C(2).[10] Therefore, the peak at 100.2 ppm is unequivocally assigned to C(1) from Table 1. The doublets at 76.1 ppm might be due to C(2), following comparison with other aldohexose phosphates.[10] The doublets at 100.2 and 76.1 ppm indicated that C(1) and C(2) are coupled with P. The coupling constants between C(1) and P, and between C(2) and P are 5.6 and 8.2 Hz, respectively. As shown in Table 1, the ^{13}C-NMR data of the reaction product of D-glucopyranose and P_{3m} are very similar to those of β-D-glucopyranosyl 1-monophosphate, indicating that only the hydroxyl group at C(1) of the β-form of D-glucopyranose is selectively phosphorylated to give β-D-glucopyranosyl 1-triphosphate. In order to confirm this, ^{1}H- and ^{31}P-NMR measurements were also performed.

Figure 2 shows the ^{1}H-NMR spectra of the H(1) region. The doublet ($J_{12}=7.6$ Hz) of H(1) of D-glucopyranose become a triplet (4.9 ppm) due to coupling with P on phosphorylation. Selective decoupling at the H(2) position reduces the H(1) signal (triplet) to the doublet with $J_{PH}=8.1$ Hz, consistent with the J_{PH} obtained from the ^{31}P-NMR spectra. In the ^{1}H-coupled ^{31}P-NMR spectrum the quartet of the P_α atom in the triphosphate derivative is also reduced to a doublet by selective H(1) decoupling,[4] indicating that H(1) is coupled with the P_α atom as shown below.



Furthermore, the ^{1}H chemical shift value of H(1) and coupling constant J_{12} in the triphosphate derivative are very similar to those of β-D-glucopyranosyl 1-phosphate (Fig. 2(C)). Therefore, the position of the hydroxyl group in D-glucopyranose phosphorylated with P_{3m} is the C(1) of D-glucopyranose.

Table 2 lists ^{1}H-NMR data for β-D-glucopyranosyl 1-triphosphate and α- and β-D-glucopyranosyl 1-monophosphates. The J_{12} value of D-glucopyranosyl 1-triphosphate is 8.1 Hz, which is typical of β-anomers of D-aldohexose 1-monophosphates and is consistent with that of β-D-glucopyranosyl 1-phosphate. This is explained by Karplus's equation for the coupling constant of $^3J_{12}$.[11] Conformation of phosphate group in the product can also be estimated from $J_{PC(2)}$ and $J_{PH(1)}$, suggesting that rotamers, in which P is *trans* to C(2) and is *cis* to C(2), exist to a considerable extent.[11]

After a long time the signals of α- and β-D-glucopyranoses decreased in spite of the fact that only β-D-glucopyranose reacts with P_{3m} to form β-D-glucopyranosyl

Table 2. ^{1}H Chemical Shifts of Phosphorylated Product and α,β-D-Glucopyranosyl 1-Phosphate

Compound	pH	Chemical shift (ppm)							Coupling constant (Hz)		
		H(1)	H(2)	H(3)	H(4)	H(5)	H(6)	H(6′)	J_{12}	J_{P1}	J_{P2}
β-D-Glucopyranosyl 1-triphosphate	12	4.90	3.19	3.40	3.23	3.37	3.72	3.55	8.1	8.1	0
α-D-Glucopyranosyl 1-phosphate	12	5.30	3.35	3.63	3.26	3.77	3.73	3.60	3.4	7.4	1.8
β-D-Glucopyranosyl 1-phosphate	12	4.88	3.30	3.50	3.30	3.48	3.90	3.65	7.7	7.7	0

1-triphosphate. Fast exchange between α- and β-anomers takes place at pH 12. Even if α-D-glucopyranose is used as the starting material, fast mutarotation between the α (36%) and β anomers (64%) occurs to give β-D-glucopyranosyl 1-triphosphate. In conclusion, β-D-glucopyranosyl 1-triphosphate is selectivity synthesized in the reaction of D-glucopyranose with P_{3m}.

Experimental

1) Chemicals Sodium cyclo-triphosphate (P_{3m}), $Na_3P_3O_9 \cdot 6H_2O$, was prepared as described in previous papers.[3,4] α-D-Glucopyranosyl 1-phosphate, β-D-glucopyranosyl 1-phosphate, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Sigma (St. Louis, U.S.A.). Other chemicals were purchased from Wako Chemicals (Osaka, Japan).

2) NMR Measurement ^{13}C-NMR spectra with broad band decoupling and ^{1}H-NMR spectra were measured with Varian Gemini 300 and 200 instruments. Samples were dissolved in D_2O (99.9%) to avoid the effect of ^{1}H in water. DSS was used as an external standard for ^{13}C and ^{1}H spectra. ^{31}P-NMR spectra with and without broad band decoupling, were obtained with a Varian VXR-500 instrument. As an external standard, 85% H_3PO_4 was used.

3) HPLC Measurement HPLC analysis was carried out with a JASCO GULLIVER HPLC system (Tokyo, Japan) coupled with a JASCO DU-4F flow injection system to detect phosphate by a post-column reaction. A column (150 × 6.0 mm i.d.) packed with a polystyrene-based anion-exchanger (TSK gel, SAX, 10 μm, TOSOH, Japan) was used for the analysis of phosphate. The column temperature was maintained at 40 °C. A convex gradient elution technique using 0.2 and 0.45 mol·dm^{-3} potassium chloride solutions was employed for the analysis of phosphate. Determination of phosphorus was carried out by spectrophotometry of a phosphorus-molybdenum heteropoly blue complex at 830 nm. The flow rate of the eluent was 1.0 ml·min^{-1}.

Acknowledgement The authors thank assistant professor M. Sugiura of Kobe Pharmaceutical University for the measurement of ^{31}P-NMR spectra.

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