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Phosphorylation of Glucose with *cyclo*-Triphosphate

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α-D-glucose reacted with *cyclo*-triphosphate (P_{3m}) to give tri-, di-, and monophosphate derivatives. The main product, glucose triphosphate was a mixture of glucose 1- and 2-triphosphates (maximum yield = about 47%). Glucose was most effectively phosphorylated in alkaline solutions (pH 10——12) at room temperature.

Much attention has recently been focused upon *cyclo*-triphosphate (P_{3m}) as a phosphorylating agent of organic compounds and a condensing agent of amino acids. P_{3m} readily reacts with ammonia,[1,2] amines,[3,4] amino acids,[5-7] alcohols,[8] phenols,[9] and nucleosides[10-15] to give phosphate derivatives. The present authors established a novel synthetic method of glucose triphosphate (glucose 1- and 2-triphosphates) by the reaction of α-D-glucose with P_{3m}. Glucose di- and monophosphates were also obtained by the reaction.

The phosphorylation reaction was examined by varying the molar ratio of P_{3m} to α-D-glucose (0.5:0.5 mol dm^{-3}—— 0.25:2.5 mol dm^{-3}), pH (7——14), and

temperature (room temperature to 70 °C). Both ^{31}P-NMR technique and HPLC - FIA (flow injection analysis) system have been applied to determine the structures and the amounts of the products.

Figure 1 shows a representative HPLC profile for the products of P_{3m} with α-D-glucose after 1 h. In addition to the peaks due to ortho- (P_1), pyro- (P_2), tri- (P_3), and cyclic triphosphate (P_{3m}), four peaks of unknown compounds (designated as Compounds A, B, C, and D) were observed at 33.2, 16.3, 7.9, and 6.1 min, respectively. By comparing the change in the peak areas with time, it was found that the yield of Compound A increased drastically to reach a maximum value of ca. 47% (as P) after 7 h, and then began to decrease gradually. The amounts of Compounds B, C, and D were small compared with Compound A, though they increased with the progress of the reaction.

The ^{31}P-NMR spectra of the reaction products shown in Fig. 2 support elucidation of the structures of the unknown compounds. By comparing ^{1}H-decoupled and ^{1}H-coupled spectra, we can discriminate phosphorylated glucose compounds from inorganic phosphates (P_1, P_2, P_3, and P_{3m}).

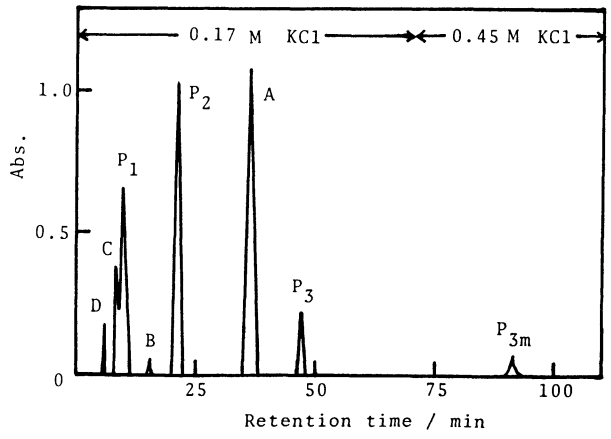

Fig. 1. Elution pattern of the reaction products of α-D-glucose with *cyclo*-triphosphate.
Glu:P_{3m} = 2.5:0.25 mol dm^{-3}; pH 12; at room temperature

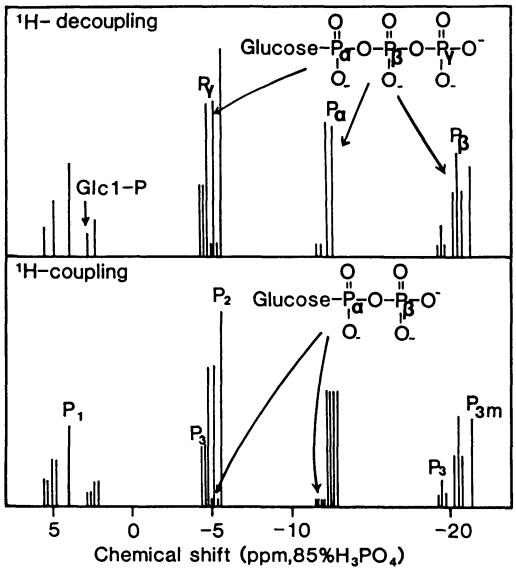

Fig.2. ^{31}P-NMR spectra of the reaction products of glucose with P_{3m}.
Glu : P_{3m} = 0.5 : 0.5 mol dm^{-3} ; pH 12 ; at room temperature ; after 5h

The peaks at -12.3 ppm (doublet), -21.3 ppm (triplet), and -5.0 ppm (doublet) were assigned to P_α, P_β, and P_γ atoms of the triphosphate derivatives of glucose, respectively. The doublet of P_α atom in the ^{1}H-decoupled spectrum was split into double doublets in the ^{1}H-coupled spectrum, showing that one proton exists near the P_α atom. It is impossible to define the position of the

triphosphate group introduced to the glucose molecule by the ^{31}P-NMR results. Possible compounds are, therefore, glucose 1-, 2-, 3-, and 4-triphosphates. It has been shown that glucose 1-monophosphate was not phosphorylated by P_{3m}, whereas glucose 6-monophosphate was easily phosphorylated.[16] Also, glucosamine is known to react with P_{3m} to give a mixture of glucosamine 1-triphosphate and 2-triphosphoramidate.[16] From these additional experimental results, Compound A is expected to be a mixture of glucose 1- and 2-triphosphates.

Two doublets at -11.8 ppm and -5.1 ppm in the ^{1}H-decoupled spectrum were attributed to P_{α} and P_{β} atoms of the diphosphate derivative of glucose. The small peak (Compound B) at 12.8 min in the HPLC profile corresponds to glucose diphosphate. By comparing ^{31}P-NMR spectra of the authentic sample, a singlet 3.5 ppm was assigned to that of glucose 1-monophosphate. Three singlets at 5.3, 5.0, and 3.3 ppm in the ^{1}H-decoupled spectrum were also attributed to monophosphate derivatives. Compounds C and D, which appeared in the HPLC profile, therefore, correspond to monophosphate derivatives or their mixtures. A further precise study is now in progress in order to clarify the structures of Compounds C and D.

Table1. Phosphorylation of glucose with cyclo-triphosphate

Reaction conditions			Yield/%		
Molar ratio (P_{3m}:Glu)	pH	Temp /°C	P_3-derivative	P_1-derivative (D)	P_1-derivative (C)
1 : 1	12	room temp	16.0	0.2	4.3
	10	room temp	20.0	0.2	1.1
1 : 5	12	room temp	47.0	1.9	6.7
		70	23.9	0.6	4.7
	10	room temp	32.0	0.7	1.3
1 : 10	12	room temp	44.3	2.0	6.4
		30	38.7	1.3	7.0
	10	room temp	32.2	0.9	1.3

The yields of the phosphorylated glucose compounds (glucose triphosphate and glucose monophosphate) determined by the HPLC analysis are summarized in Table

1. The yields of glucose diphosphate were negligibly small, and are not shown in the table.

Optimum conditions for the phosphorylation of glucose with P_{3m} were the mixing ratio of 5:1 — 10:1, pH 12, and at room temperature.

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