SYNTHESES OF ORGANIC PHOSPHORUS COMPOUNDS WITH -NH-P-NH- AND -NH-P-O- BONDS BY INORGANIC MONOIMIDO-CYCLO-TRIPHOSPHATE

HIDEKO INOUE¹, HIROKAZU NAKAYAMA¹, HIROYUKI NARIAI²,
MITSUTOMO TSUHAKE^{1*}

¹Department of Functional Molecular Chemistry, Kobe Pharmaceutical
University, 4-19-1 Motoyamakita-machi, Higashinada, Kobe 658-8558 Japan.

²Faculty of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada, Kobe
657-8501 Japan.

Abstract The phosphorylation of amino acids with monoimido-*cyclo*-triphosphate proceeded on only amino groups. The phosphorylation products of glycine, α -alanine, and valine were *N*-(carboxymethyl)diphosphoramidophosphoramidate, *N*-(1-carboxyethyl)diphosphoramidophosphoramidate, *N*-(1-carboxy-2-methylpropyl)diphosphoramidophosphoramidate with their yields of 32, 17, and 12 %, respectively. On the other hand, in the phosphorylation of β -alanine and γ -aminobutyric acid, *N*-(2-carboxyethyl)diphosphoramidophosphoramidate and *N*-(2-carboxyethyl)phosphoramidodiphosphoramidate for β -alanine, and *N*-(3-carboxypropyl)diphosphoramidophosphoramidate and *N*-(3-carboxypropyl)phosphoramidodiphosphoramidate for γ -aminobutyric acid with their total yields of 57 and 64 %, respectively were obtained.

INTRODUCTION

Sodium *cyclo*-triphosphate, $\text{Na}_3\text{P}_3\text{O}_9$ ($\text{P}_{3\text{m}}$), is a very attractive phosphorylating agent for synthesizing biologically important organic phosphorus compounds in one step in aqueous solution. Previously, we reported that the alkylamines¹, aminoalcohols², and carbohydrates³⁻⁸ are readily phosphorylated with $\text{P}_{3\text{m}}$ to give their triphosphate derivatives. On the other hand, monophosphate derivatives⁹ of amino acids are also obtained by the reaction of amino acids with $\text{P}_{3\text{m}}$.

Recently, we demonstrated that the phosphorylation of methylamine¹⁰ proceeded with monoimido-*cyclo*-triphosphate, $\text{Na}_3\text{P}_3\text{O}_8\text{NH}$ (MCTP), in which one of oxygen atoms constituting $\text{P}_{3\text{m}}$ ring was exchanged by nitrogen atom. And the phosphorylation products were diphosphoramidophosphonomethylamine and phosphoramidodiphosphonomethylamine with P-N bond. More recently, mono-, di-, and oligosaccharides were found to be phosphorylated by MCTP to give monoimidotriphosphate esters¹¹. We have therefore explored the application of

MCTP for phosphorylation of organic compounds.

In this study, the phosphorylation of amino acids by MCTP was studied to develop new phosphorylating agent in aqueous solution and to synthesize the novel organic phosphorus compounds with P-N bond.

RESULTS AND DISCUSSION

Reaction of amino acids and MCTP

The phosphorylation of glycine, α -alanine, or valine with MCTP gave a product by HPLC and the products were named **1**, **2**, and **3**, respectively. On the other hand, β -alanine or γ -aminobutyric acid showed two products and the products were named **4** and **5** for β -alanine and **6** and **7** for γ -aminobutyric acid, respectively. From the retention time of HPLC, all the products were assigned to monoimidotriphosphate derivatives of amino acids. A previous work showed that the reaction products of glycine, α -alanine, or valine with P_{3m} were monophosphate derivatives⁹ and those of β -alanine and γ -aminobutyric acid were triphosphate derivatives. These results suggested that monoimidotriphosphate derivatives with -P-NH- linkage are stable in alkaline solution and are difficult to hydrolyze compared with triphosphate derivatives with -P-O- linkage. Table 1 summarizes the yields of phosphorylation products of amino acids (1.5 M) with MCTP (0.3 M) at pH 12 and 25 °C. The maximum yields of products **1**, **2**, and **3** were 32, 17, and 12 %, respectively, and the total yield for **4** and **5** was 57 %, and that for **6** and **7** was 64 %, respectively.

Table 1 Yields of the phosphorylated products of amino acids (1.5 M) with MCTP (0.3 M) at pH 12 and 25 °C

Product	Time (d)	Yield (%)
1	15	32
2	11	17
3	15	12
4	15	41
5	20	16
6	16	46
7	21	18

Structures of the phosphorylation products of amino acids

To identify the reaction products of β -alanine and MCTP, ^{31}P and ^1H NMR spectra were measured. Figure 1 shows the ^{31}P NMR spectra of the reaction solution, indicating that the products are two monoimidotriphosphate derivatives. The peaks at 7.0, -9.1, and -4.6 ppm of the ^{31}P NMR spectra were assigned to the product **4**, and the peaks at 0.37, -6.5, and 0.7 ppm to the product **5**, respectively, by comparing the integration areas of the NMR signals of two monoimidotriphosphate derivatives with the peak areas of HPLC profile. The ^1H non-decoupling spectrum had two doublet of doublet of doublets (7.0 and 0.37 ppm), which became two doublets on ^1H decoupling, corresponding the characteristic peak of P_{α} in triphosphate derivatives of amino acids⁹ and aminoalchols².

Figure 1 ^{31}P NMR spectra of the reaction solution
MCTP : β -alanine = 0.3 M : 1.5 M, pH 12, and 25 $^\circ\text{C}$, after 17 d

Figure 2 ^{31}P - ^1H 2D NMR spectra of products in the
reaction solution
MCTP : β -alanine = 0.3 M : 1.5 M, pH 12, 25 $^\circ\text{C}$, after 17 d.

The doublets at -4.6 and 0.7 ppm were assigned to the end phosphorus atom (P_γ). The other doublet of doublets at -9.1 and -6.5 ppm are characteristic of the middle phosphorus atom (P_β) of monoimidotriphosphate¹⁰. Compared with the reaction products of amino acids⁹ and aminoalcohols² with P_{3m} , **4** and **5** caused significant down-field shifts in the ^{31}P NMR spectra due to a nitrogen atom.

As shown in Fig. 2, the ^{31}P - ^1H heteronuclear multiple bond correlation spectroscopy (HMBC) 2D-NMR spectrum showed a correlation of P_α at 7.0 ppm and H-1 signal at 2.93 ppm. The doublet at 2.93 ppm could be assigned to CH_2 of **4**. The down-field shift³⁻⁸ from 2.70 ppm (CH_2 of β -alanine) to 2.93 ppm indicated the phosphorylation of β -alanine with MCTP. The $^3J_{P_\alpha, H}$ value was 7.7 Hz, which is consistent with that obtained from the ^{31}P NMR data.

Figure 3 shows the structures of phosphorylation products in the reaction of β -alanine with MCTP. Compared with triphosphate^{2,9}, the chemical shifts of P_α and P_β of **4** indicated significant down-field shifts in the ^{31}P NMR spectra, whereas there was no shift for P_γ . Therefore, the product **4** was verified to be *N*-(2-carboxyethyl) diphosphoramidophosphoramidate.

The peaks at 0.37 , -6.5 , and 0.7 ppm in the ^{31}P NMR spectra (Fig. 1) were due to the product **5**. The ^1H decoupling spectrum showed a doublet at 0.37 ppm, which became doublet of doublet of doublets on ^1H non-decoupling spectrum. Thus, the doublet at 0.37 ppm is assigned to P_α of **5**. The doublet of doublets at -6.5 ppm is P_β of **5**. The doublet at 0.7 ppm is assigned to P_γ of **5**. Compared with triphosphate derivatives^{2,9}, the chemical shifts of P_β and P_γ of **5** indicated significant down-field shifts in the ^{31}P NMR spectra, whereas there was no shift for P_α . A correlation between P_α at 0.37 ppm and the ^1H signal at 2.97 ppm is shown in Fig. 2. The $^3J_{P_\alpha, H}$ value was 9.0 Hz, which is consistent with that obtained from the ^{31}P NMR data. Therefore, the product **5** was assigned to *N*-(2-carboxyethyl)phosphoramidodiphosphoramidate (Fig. 3).

Figure 3 Structures of **4** and **5**

The products **1**, **2**, **3**, **6**, and **7** were identified similar to the cases of **4** and **5**. The phosphorylation products were verified to be *N*-(carboxymethyl) diphosphoramidophosphoramidate (**1**), *N*-(1-carboxyethyl) diphosphoramidophosphoramidate (**2**), *N*-(1-carboxy-2-methylpropyl) diphosphoramidophosphoramidate (**3**), *N*-(3-carboxypropyl) diphosphoramidophosphoramidate (**6**), and *N*-(3-carboxypropyl) phosphoramidodiphosphoramidate (**7**), respectively.

Reaction mechanism of amino acids with MCTP

The phosphorylation of β -alanine with MCTP is explained by scheme 1. At pH 12, MCTP is easily attacked by nucleophilic reagents such as methylamine¹⁰ and cyclodextrin¹¹. In the present study, the lone electron pair on the amino group of amino acid nucleophilically attacks a phosphorus atom on MCTP to open its six-membered ring. The phosphorylation of glycine, α -alanine, and valine proceeded on only phosphorus of -O-P-NH- linkage. On the other hand, the phosphorylation of β -alanine and γ -aminobutyric acid proceeded on not only phosphorus of -O-P-NH- linkage, but also phosphorus of -O-P-O- linkage. The values of pK_2 of glycine, α -alanine, valine, β -alanine and γ -aminobutyric acid are 9.57, 9.69, 9.49, 10.10, and 10.28, respectively. In general, the reactivity for the phosphorylation of amino acids with MCTP increased with an increase in pK_2 value of the amino group.

Therefore, the amino acids with $pK_2 > 10$ would be reactive with phosphorus atom of both -O-P-NH- and -O-P-O- linkage. No attack by the carboxyl group of amino acid would be due to the electric repulsion between carboxylate ion and MCTP.

Scheme 1 Reaction mechanism of β -alanine with MCTP

EXPERIMENTAL

Monoimido-*cyclo*-triphosphate, $\text{Na}_3\text{P}_3\text{O}_8\text{NH}$ (MCTP), was prepared according to a previous paper¹². Other reagents were purchased from Wako Chemicals Co. Ltd. They were all guaranteed reagents and were used without further purification.

The reaction of amino acids (1.5 M) with MCTP (0.3 M) was carried out at pH 12 by adding 6 M sodium hydroxide solution and 25 °C. The separation of 1-7 from the reaction solution were accomplished by anion-exchange chromatography with 2×80 cm column filled with Dowex 1-X2 resin (100-200 mesh, chloride form). Elution was carried out with 0.3 M KCl aqueous solution, and each 50 ml fraction was measured by HPLC. The solution fractionated was concentrated at -113°C *in vacuo* (freeze-drying). For the purpose of desalting, an aqueous solution of the concentrate was passed over the PD-10 column (Amersham Biosciences, NJ, USA).

^{31}P NMR spectra with and without broad band ^1H decoupling and ^{31}P - ^1H 2D HMBC spectra were obtained with a Varian INOVA-500 spectrometer using 85 % H_3PO_4 as an external standard.

HPLC analysis was carried out with a JASCO PU-980 HPLC (Tokyo, Japan) coupled with a JASCO DU-4F flow injection system to detect phosphate by a post-column reaction. A column (150 $\times$ 6.0 mm I.D.) packed with a polystyrene-based anion-exchanger (TSK gel, SAX, 5 μm , TOSOH, Japan) was used for the analysis of phosphate. The system control, data collection, and data analysis were carried by JASCO-BORWIN system.

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