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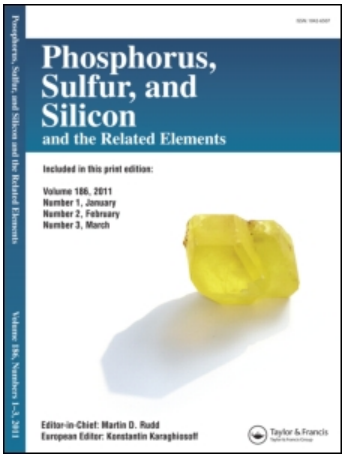

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THE BIOORGANIC CHEMICAL REACTIONS OF *N*-PHOSPHOAMINO ACIDS WITHOUT SIDE CHAIN FUNCTIONAL GROUP PARTICIPATED BY PHOSPHORYL GROUP

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THE BIOORGANIC CHEMICAL REACTIONS OF *N*-PHOSPHOAMINO ACIDS WITHOUT SIDE CHAIN FUNCTIONAL GROUP PARTICIPATED BY PHOSPHORYL GROUP

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The bioorganic chemical reactions of *N*-phosphoamino acids without a side chain functional group are complex and interesting. With the participation of the phosphoryl group, *N*-phosphoamino acids can self-activate to give the peptides, esters and ester exchange products. An intramolecular mixed phosphorylic-carboxylic anhydride intermediate may exist in these bioorganic chemical processes.

Key words: *N*-Phosphoamino acid; phosphoryl group; phosphorylic-carboxylic anhydride intermediate; NMR.

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INTRODUCTION

Phosphorus plays an important role in the chemistry of life. Acting as the skeleton, phosphorus makes up to 9% in weight of DNA. It has been confirmed that the activities of many enzymes are regulated through the phosphorylation-dephosphorylation mechanism. In almost all the metabolic processes, phosphate intermediates are involved. It is obvious that the phosphoryl group participation reaction executes the vital role. However, the intrinsic chemical relationship between the phosphoryl and the bio-molecules is still not clear.[1]

Peptide formation, esterification and ester exchange at the phosphoryl group are the fundamental chemical reaction of the amino acids during life process. Without peptide formation, enzyme formation and life process would not exist. Esterification of the amino acids is the first step of the protein biosynthesis. Without the esterification of the amino acids, they can't be activated and carried by tRNA and no protein synthesis was possible. Cech's work documented that ester exchange at the phosphoryl group is the key to the self-catalysis of the RNA and it relates to the foundation of biocatalysis.[8]

In this paper, the well designed *N*-phosphoamino acids[2–7] were used as the models to study the effects of the phosphoryl group on the bio-mimic reaction. Additionally, the study of the *N*-phosphoamino acids derived from simple amino acids could set up the foundation for understanding the mechanism of the complex naturally occurring molecules. We focused our attention on the bioorganic chemical reaction of *N*-phosphorylated amino acids without a side chain functional group.

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RESULTS AND DISCUSSION

Generally the *N*-phosphoamino acids without side chain functional groups were very stable especially as they were phosphorylated by the sterically hindered alkylphosphoryl group. However, once a less hindered phosphoryl group was introduced, many interesting bio-mimic reactions could be stimulated. If the fresh pure *N*-phosphoamino acid was incubated in alcohol at 40°C for a few hours, the spectra of ^{31}P-NMR showed complicated results (Table I). After removal of solvent, FAB-MS of the residues showed that many possible products have formed (Table II). Among them are the esterification, mono- and di-ester exchanges on the phosphoryl group and the peptides or peptide ester products. The relationship among these products is shown in Figure 1.

In order to understand the process in detail, the products resulting from the self-activation were isolated and identified. To simplify the isolation process, the *N*-dibutylphosphoamino acid was incubated in *n*-butanol, which will reduce the number of the final products, since the ester exchanges on the phosphoryl will be transferred into itself. For the purpose of suppressing the peptide formation and esterification reaction, a dilution technique was adopted in which the *N*-dibutylphosphoryl amino acid solution was added dropwise into the specified alcohol. In

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TABLE I

^{31}P-NMR data of *N*-phosphoamino acids after incubation in alcohol at 40°C for 15 h

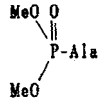
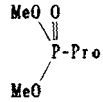
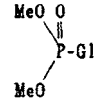
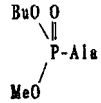
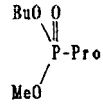
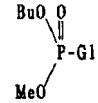
DBP-Ala	DBP-Pro	DBP-Gly
10.0 (15)	8.42 (10)	9.18 (100)
8.6 (15)	7.81 (30)	8.35 (25)
7.4 (65)	7.70 (10)	1.91 (10)
7.1 (25)	0.64 (20)	1.18 (15)
1.1 (80)	-0.58 (100)	-10.5 (10)
0.2 (25)	-10.35 (30)	
0.0 (50)		
-0.9 (45)		
-1.2 (100)		
-12.0 (60)		
-12.5 (10)		

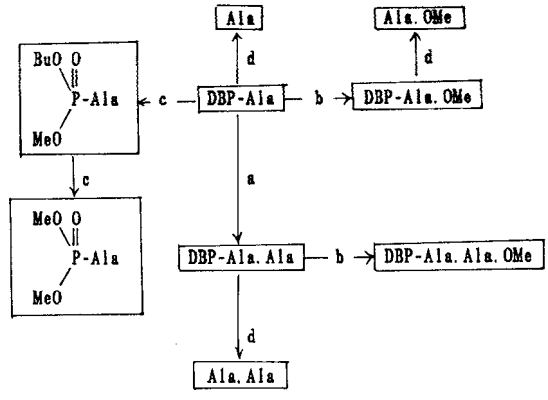
where DBP-= BuO, BuO, O, P-

The data inside the parentheses indicates the relative intensity.

TABLE II

FAB-MS data of *N*-phosphoamino acids after incubation in MeOH

DBP-Ala		DBP-Pro		DBP-Gly	
$(MH)^+$	Possible Compound	$(MH)^+$	Possible Compound	$(MH)^+$	Possible Compound
90	Ala	116	Pro	75	Gly
104	Ala. OMe			89	Gly. OMe
161	Ala. Ala	212	Pro. Pro	133	Gly. Gly
198		224		184	
236		262		222	
282	DBP-Ala	308	DBP-Pro	268	DBP-Gly
296	DBP-Ala. OMe	322	DBP-Pro. OMe	282	DBP-Gly. OMe
353	DBP-Ala. Ala	405	DBP-Pro. Pro	325	DBP-Gly. Gly
367	DBP-Ala. Ala. OMe			339	DBP-Gly, Gly. OMe



a: Peptide formation

b: Esterification

c: Ester exchange at phosphoryl

d: Dephosphorylation

FIGURE 1 The relationship among the self-activating products of DBP-Ala after incubation.

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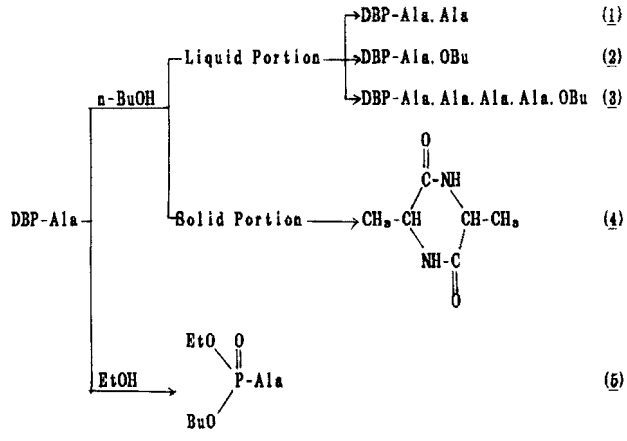

FIGURE 2 Isolation of the products from the DBP-Ala incubated mixture.

TABLE III

IR, ^{31}P-NMR and FAB-MS of the products isolated

Product	IR (cm^{-1})	^{31}P-NMR (ppm)	FAB-MS $(MH)^+$
(1)	1735 (s)	7.43	353
	1640 (s)		
(2)	1750 (s)	7.11	338
(3)	1730 (s)	7.09	551
	1660 (s)		
(4)	1675 (s)	none	143
(5)	1750 (s)	7.46	254

TABLE IV

FAB-HRMS of the products isolated

Product	Molecular Formula	$(MH)^+/Z$ Calculated Mass	$(MH)^+/Z$ Observed Mass	Notes
(1)	$C_{14}H_{29}O_6N_2P$	353.1842	353.1873	
(2)				
(3)	$C_{24}H_{47}O_8N_4P$	551.3210	551.3208	
(4)	$C_8H_{10}O_2N_2P$	143.0821	143.0842	
(5)	$C_8H_{21}O_6NP$	254.1157	254.1165	

*All ^{1}H-NMR, ^{31}P-NMR, ^{13}C-NMR, FAB-MS and IR data have been compared with the known data in Reference 3.

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TABLE V

^{1}H-NMR and ^{13}C-NMR of the products isolated

Product	^{1}H-NMR (ppm)	^{13}C-NMR (ppm) ($^nJ_{P-C}$ (Hz))						
		$(n\text{-BuO})_2\text{-}\overset{\overset{O}{\|}}{P}\text{-NH-}\overset{\overset{R_1}{\|}}{C}_{\alpha 1}\text{H-}\overset{\overset{O}{\|}}{C}\text{-NH-}\overset{\overset{R_2}{\|}}{C}_{\alpha 2}\text{H-COOR}_3$						
		C-N	COR_3	$C_{\alpha 1}$	R_1	$C_{\alpha 2}$	R_2	R_3
(1)	0.83-1.00 (m, 6H) 1.35-1.67 (m, 14H) 2.84-2.95 (m, 2H) 3.98-4.18 (m, 2H) 4.18-4.21 (m, 4H) 9.4 (s, 1H)	174.2	173.3	66.3	18.8	42.9	18.5	
(2)	0.66-0.84 (m, 9H) 1.23-1.37 (m, 16H) 3.51-4.15 (m, 8H)		172.1	66.9	17.0			11.8 17.2 18.6 62.9
(5)	0.60-0.92 (m, 6H) 1.00-1.32 (m, 7H) 1.80-2.00 (s, 1H) 3.32-3.64 (m, 4H) 3.64-4.16 (m, 1H) 7.56-8.55 (s, br, 1H)	$(R_3O)(R_3O)\text{-}\overset{\overset{O}{\|}}{P}\text{-NH-}\overset{\overset{R_1}{\|}}{C}_{\alpha}\text{H-}\overset{\overset{O}{\|}}{C}\text{-OH}$						
		COOH	C_α	R_1	R_2	R_3		
		173.4 (7.8)	57.4	22.4	13.7 61.8	13.6 17.1 18.7 66.1		

this way the corresponding products were isolated (Figure 2) and their structures were determined by spectral analysis (Tables III, IV and V).

The free amino acids are relatively inactive. However, once a phosphoryl group was introduced many interesting bioorganic chemical reactions occurred without the help of any other activators. These self-activating reactions are novel phenomenon. It was also found that if the carboxyl group was esterified (e.g. DBP-Gly.OEt) or the phosphoryl group was replaced by another *N*-protecting group (such as Boc-, Z- etc.), no self-activation occurred. The size of the dialkylphosphoryl group (dibutylphosphoryl-, DBP-, diisopropylphosphoryl-, DIPP-) causes a significant effect. These facts indicated that the phosphoryl and free carboxylic group must participate in the self-activation process. The reaction may pass through a crowded intermediate which is greatly affected by the steric size of the nearby group. Based upon these experimental results, it appears that an intramolecular mixed phosphorylic-carboxylic anhydride intermediate may exist in the reaction process. In this intermediate, the carboxylic group and phosphorylic group activate each other and the attack of the nucleophilic reagents (such as alcohol, amine etc.) becomes facile. All three kinds of self-activation products, namely, peptide, ester and ester exchange on phosphoryl can be inferred from it (Figure 3).

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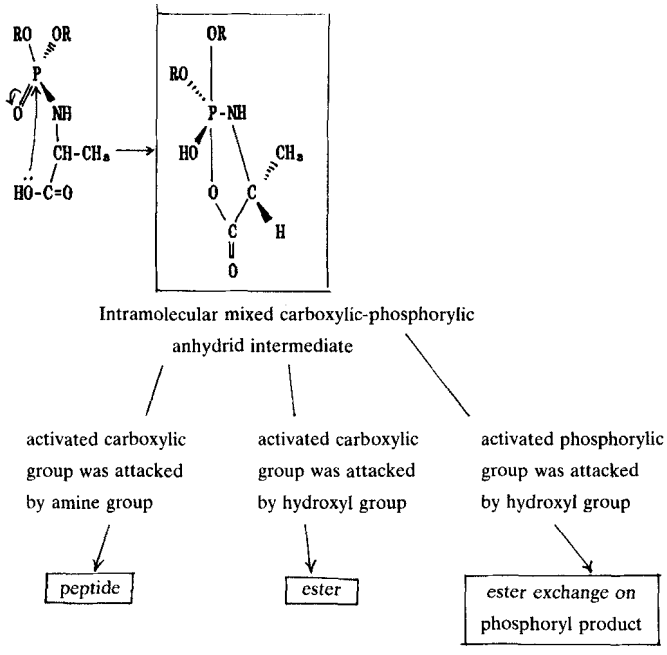

FIGURE 3 *Intramolecular mixed phosphorylic-carboxylic anhydride intermediate.*

CONCLUSION

N-phosphoamino acids without side chain functional group can activate themselves to have many bioorganic chemical reactions occur, for example, peptide formation, esterification and ester exchange on phosphoryl. Phosphoryl group participation is the key to these reactions. An intramolecular mixed carboxylic-phosphorylic anhydride intermediate is suggested as an intermediate during this process.

EXPERIMENTAL

Methods. ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra were recorded on a JEOL FX-100 spectrometer, Bruker AM-300 spectrometer or JEOL FX-90 spectrometer, chemical shifts of ^{1}H-NMR are reported in parts per million relative to internal tetramethylsilane (0.00 ppm). The ^{13}C-NMR spectra used chloroform as the internal reference at 76.9 ppm. 85% H_3PO_4 was used as the external standard for ^{31}P-NMR spectra. Positive-ion FAB-MS data were obtained on a KYKY fast-atom gun. IR spectra were measured as KBr plates or liquid film on KBr on a Shimadzu IR-408 spectrometer. Thin-layer chromatography using 10–40 μm silica gel was made in the Ocean Chemical Factory, Qingdao, China.

Preparation. The preparation of *N*-(dibutylphosphoryl)amino acids (DBP-Ala, DBP-Pro, DBP-Gly), the preparation of *N*-(diisopropylphosphoryl) amino acids (DIPP-Ala, DIPP-Pro, DIPP-Gly) and the preparation of *N*-(dibutylphosphoryl) glycine ethyl ester (DBP-Gly.OEt) were carried out according to the literature.[2,3] All physical constants and spectroscopic data of the products agree with the literature values.

Isolation of the peptide and ester. To about 3.0 g of fresh pure smple, 10 mL of *n*-butanol was added. The mixture was stirred at 40–50°C for 15 hours.

The liquid portion and the solid portion were separated carefully. The *n*-butanol in the liquid portion was removed by distillation in vacuo. Vacuum liquid chromatography (VLC) was used, which is the most suitable for the gradient elution. The apparatus and technique are described clearly in the liter-

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ature.[9,10] Column parameters: diameter 4.5 cm; height 7.0 cm; fritted disk's ASTM 10–20 μm; absorbent silica gel, 10–40 μm vacuum: water aspirator, 20–60 mmHg; solvent mixtures: changed from petroleum ether, chloroform to ethyl acetate on a gradient. The rate of the polarity change depended on the thin-layer chromatography analysis of each fraction collected.

The solid portion must first be washed with petroleum ether thoroughly and then dried in vacuo. Vacuum liquid chromatography (VLC) was then used. Column parameters: diameter 2.8 cm; height 5.5 cm; fritted disk's ASTM 10–20 μm; absorbent silica gel, 10–40 μm; vacuum water aspirator, 20–60 mmHg; solvent mixtures: changed from methyl alcohol to ethyl alcohol on a gradient. The rate of the polarity change depended on the thin-layer chromatography analysis of each fraction collected.

Isolation of the ester exchange on phosphoryl product. The ethyl alcohol was pre-warmed to 40°C and was maintained at that temperature. The phosphoamino acid/acetate solution was added dropwise in 4 hours, and then stirred at the same temperature for another 4 hours. The mixture was treated as the liquid portion described previously.

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