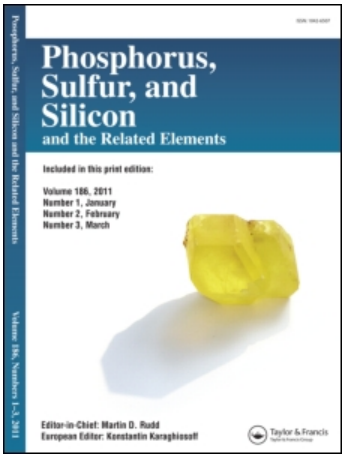

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Mechanism study on the Oligomerization of Amino Acids into Peptides by Phosphorus Trichloride

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As treated by phosphorus trichloride, amino acids could oligomerize into polypeptides. Based on the results obtained by ^{31}P-NMR and ESI-MS/MS, a possible reaction mechanism was proposed. The mechanism might undergo a penta-coordinated phosphorus intermediat. The activated amino acid was a five-membered cyclic penta-coordinated phosphorus intermediate. The nucleophilic attack of the amino group from an amino acid or peptide on the carbonyl group of intermediate led to the formation of peptide and released one equivalent dichloride phosphoric acid. The repetition of the reaction sequence generated a series of oligopeptides.

Keywords Mechanism; penta-coordinated phosphorus; peptides; phosphorus trichloride

INTRODUCTION

More and more experimental results implied that phosphorus might play an important role in the formation of peptides in a prebiotic condition. The polymerization of free and substituted amino acid phosphor-anhydrides, which are energy-rich monomers that permit the formation of peptide bonds, were studied.[1–4] Rabinowitz[5] proposed a phosphoryl group intermediate mechanism when glycine and alanine were treated with cyclic polyphosphate and dipeptides were obtained. Baba and Tsuhako[6,7] studied the reaction with high performance liquid chromatography (HPLC) and ^{31}P-NMR, and a five-membered cyclic carboxylic acid-phosphoric acid mix anhydride intermediate was proposed. *N*-phosphoryl α-amino acids and *N,O*-bis(trimethylsilyl)-α-amino acids

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could self-activate to give peptides, and the reaction mechanism underwent a five-membered cyclic penta-coordinated phosphoric-carboxylic mixed anhydride intermediate.[8–10] Our recent study showed that α-amino acids could be assembled into homopeptide libraries with the assistance of phosphorus oxychloride or phosphorus pentachloride.[11,12] We report herein that α-amino acids treated with phosphorus trichloride can oligomerize into polypeptides in polar aprotic solvent. The reaction mechanism was studied by ^{31}P NMR and electrospray ionization mass spectrometry (ESI-MS/MS), and a five-membered cyclic penta-coordinated phosphorus intermediate of amino acid was proposed.

EXPERIMENTAL

General Preparation of Oligopeptides

5.0 mmol amino acids were mixed with 5.0 mmol phosphorus trichloride in 15 mL acetonitrile and stirred at a proper temperature. The reaction was monitored by ^{31}P NMR. Another parallel experiment was quenched by adding 10 mL water or alcohol to the reaction mixture, which was determined by ESI-MS/MS.

Mass Spectrometry

Mass spectra were performed on a Bruker Esquire 3000 plus ion trap spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to m/z 6000. The samples were dissolved in water or alcohols were ionized by electrospray ionization (ESI) and continuously infused into the ESI chamber at a flow rate of 4 L/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Co.). Ions were gated into the ion trap for scan by using injection times of 50 ms. All the experiments were acquired in positive ion mode.

^{31}P NMR

^{31}P NMR spectra were performed on a Bruker DPX-400 spectrometer, using 85% H_3PO_4 as an external standard. ^{31}P-NMR spectra were performed on a Bruker AC 200 p spectrometer for *N*,*O*-bis(trimethylsilyl)-alanine reacted by phosphorus trichloride, using 85% H_3PO_4 as an external standard.

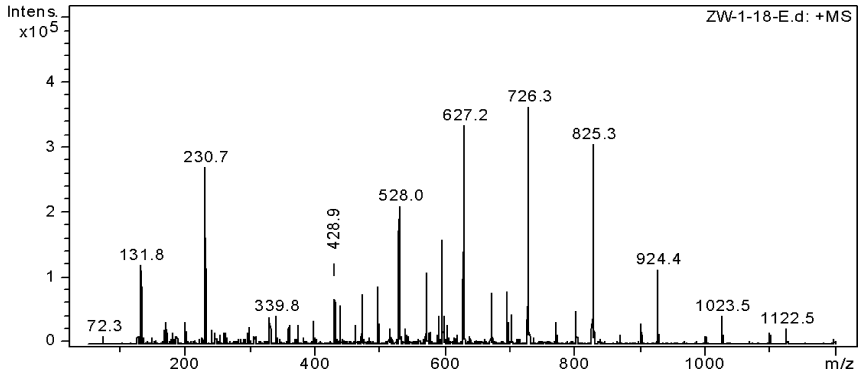


FIGURE 1 Positive ESI-MS spectrum of peptides from phosphorus trichloride reacting with L-Val for 48 h and then quenched with methanol.

RESULTS AND DISCUSSION

Analysis of the Oligomers

After quenching the reaction of L-valine (L-Val) with phosphorus trichloride by H_2O or various alcohols, the corresponding peptides and peptide esters were obtained, respectively. The structures of these products were confirmed by ESI-MS/MS. Figure 1 shows a series of mass peaks (m/z 132+99n, n = 1–10) corresponding to oliogmeric products of the reaction of L-Val with phosphorus trichloride for 48 h quenched with methanol. We found that not only the amounts but the length of the peptides increased with prolonged reaction time. Oligopeptides that were formed for other amino acids were also determined by positive ion ESI-MS and the results are listed in Table I. The protonated molecular ions corresponding to oligopeptides from monomer to nonamer were observed.

Mechanism of Amino Acids Mediated Oligomerization into Peptides by Phosphorus Trichloride

The ESI-MS/MS results described previously show that the length of the peptides was dependent on reaction time. Amino acids could hardly form any peptide in the absence of phosphorus trichloride at room temperature, which indicated that phosphorus trichloride must be participated in these reactions. ^{31}P NMR spectroscopy is one of the most important tools for monitoring the reaction mechanistic course of phosphorus compounds, and it can be used to investigate the chemical and stereochemical behavior of reaction intermediates that are difficult or even

TABLE I ESI-MS Analysis of the Peptides from the Reaction of Amino Acids with PCl_3 for 48 h Followed by Quenching with H_2O or Alcohols

		m/z (relative intensity,%)								
Quenching reagent	Products	1	2	3	4	5	6	7	8	9
H_2O	$(Val)_nOH$	118(10)	217(43)	316(11)	415(24)	514(45)	613(52)	712(47)	811(25)	910(4)
$(Leu)_nOH$	132(13)	245(27)	358(9)	471(20)	584(41)	697(50)	810(43)	923(18)	1036(7)	
$(Ala)_nOH$	90(9)	161(40)	232(12)	303(22)	374(45)	445(55)	516(42)	587(26)	658(15)	
MeOH	$(Val)_nOMe$	132(11)	231(43)	330(11)	429(20)	528(19)	627(18)	726(29)	825(26)	924(12)
$(Leu)_nOMe$	146(2)	259(6)	372(11)	485(11)	598(35)	711(64)	824(46)	937(30)	1050(11)	
	$(Ala)_nOMe$	104(3)	175(20)	246(9)	317(4)	388(9)	459(16)	530(21)	601(31)	672(15)
EtOH	$(Val)_nOEt$	146(80)	245(100)	344(23)	443(42)	542(67)	641(45)	740(66)	839(45)	938(10)
^{i}PrOH	$(Val)_nOPr^i$	160(43)	259(100)	358(12)	457(20)	556(30)	655(32)	754(22)	853(11)	952(3)

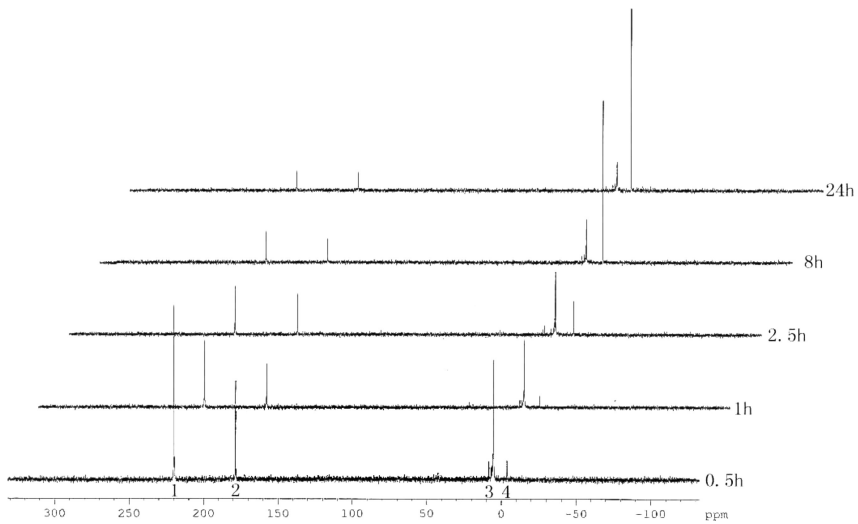
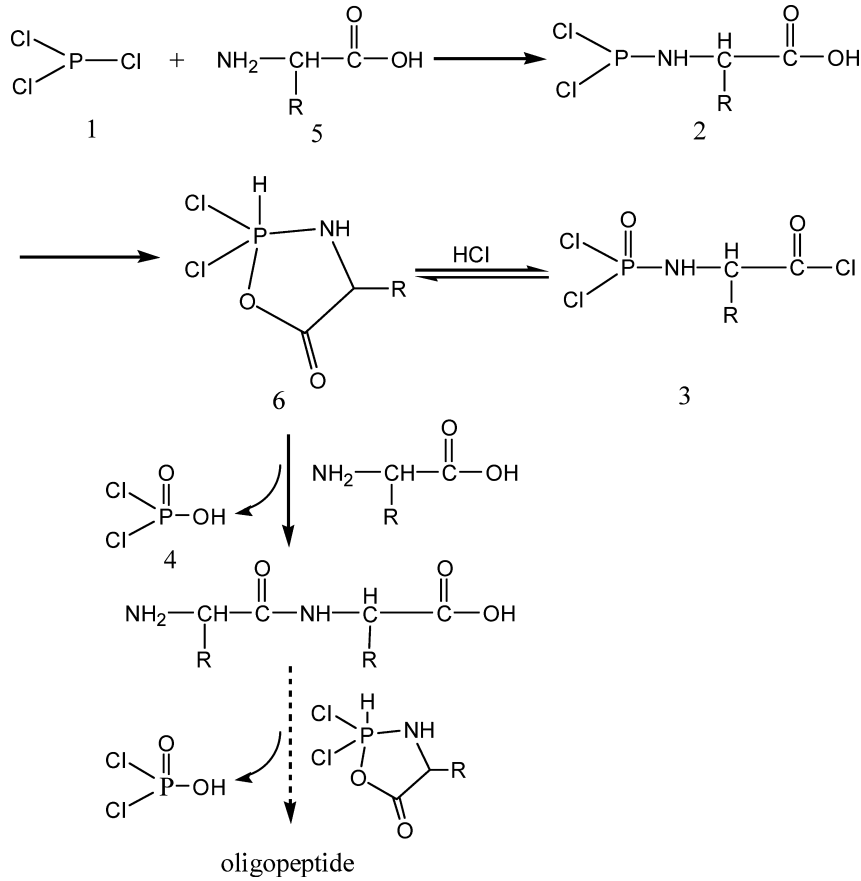


FIGURE 2 The stack ^{31}P NMR spectra of L-Val oligomerization into peptides mediated by phosphorus trichloride.

impossible by isolation. The progress of the reaction was monitored by ^{31}P NMR spectroscopy, as is shown in Figure 2.

At the beginning, the starting material 1 (δ 220) quickly reacted with amino acids and **2** (δ 178), **3** (δ 5.38), and **4** (δ–3 to −7) were obtained. **1** and **2** are tri-coordinated phosphorus compounds, while **3** and **4** are tetra-coordinated phosphorus compounds. As the reaction continued, the amount of **1**, **2**, and **3** gradually decreased while the amount of **4** increased, as is shown in Figure 2.

^{31}P NMR Stack results seem to indicate a possible mechanism, as shown in Scheme 1. The reaction of amino acids with phosphorus trichloride led to **2**, then **2** isomerized into a penta-coordinated phosphonic-carboxylic mixed anhydride **6**. Anhydride **6** was considered to be the activated amino acid. Nucleophilic attack of the secondary amino group from another amino acid at the carbonyl of **6**, dipeptide was formed and one *equivalent* dichloride phosphoric acid molecule left. Repetition of this sequence of reactions generated successively longer peptides. The oligomerization reaction continues until the activated amino acids were used up. **2** could also reversibly covert to **3**. As shown in Figure 3, trimethyl phosphate and *N-(O,O*-bimethyl)-phosphoryl-valine methyl ester were observed in a positive ESI-MS spectrum of phosphorus trichloride reacting with L-Val for 1 h at 0°C then quenched with methanol, which is consistent with the conclusion



SCHEME 1 The possible mechanism of self-assembly into peptide for amino acids mediated by phosphorus trichloride.

that the tetra-coordinated phosphorus compound **3** was obtained. The mass number at m/z 141 corresponds to trimethyl phosphate and m/z 240 corresponds to *N-(O,O*-bimethyl)-phosphoryl-valine methyl ester; thestructure was conformed by MS/MS. The protonated *N-(O,O*-bimethyl)-phosphoryl-valine methyl ester could yield ions $[M+1–32]^+$, $[M+1–28]^+$ by stepwise loss of methanol and carbon monoxide. The amount of **3** gradually decreased, which indicated that **3** participated the formation of peptides, but **3** could not be a nucleophilic attacked by another amino acid directly. These results implied that there might exist an equilibrium between **2** and **3**. Among these steps, the activation of the amino acid is the key step. However, the penta-coordinated

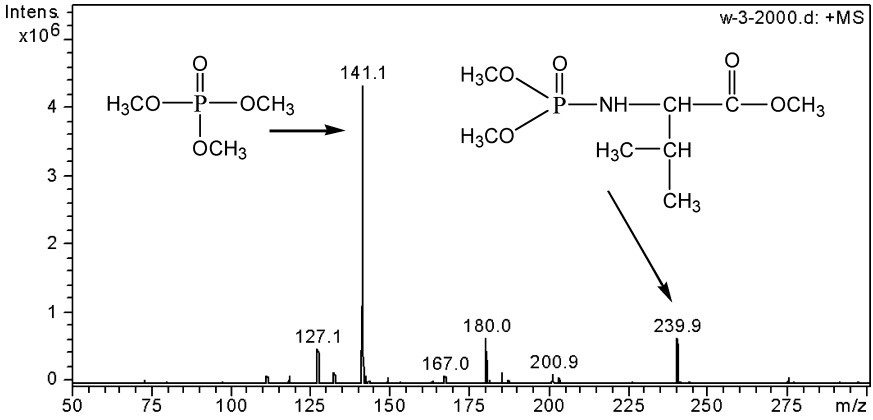


FIGURE 3 Positive ESI-MS spectrum of L-Val reacting with phosphorus trichloride for 1h at 0 then quenched with methanol.

phosphorus intermediate is unstable and only exists as instantaneous intermediate. There is no signal of penta-coordinated phosphorus intermediate appeared in the stack ^{31}P NMR spectra. The process of *N,O*-bis(trimethylsilyl)-α-alanine reacting with phosphorus trichloride in dichloromethane was monitored and the signal of penta-coordinated phosphorus intermediate (δ -68.5, δ -68.3) appeared in ^{31}P NMR spectroscopy, as is shown in Figure 4.

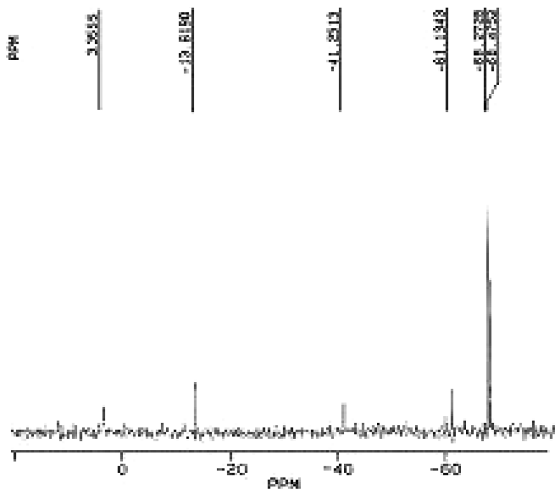


FIGURE 4 The ^{31}P NMR spectrum of penta-coordinated phosphorus from *N,O*-bis(trimethylsilyl)-α-alanine reacting with phosphorus trichloride.

The proposed mechanism that only amino acids, mediated by phosphorus trichloride, could form five-membered cyclic phosphonic-carboxylic anhydride and assemble into peptides suggests that phosphorus should play important roles in the prebiotic synthesis of polypeptide and biosynthesis of protein. On the other hand, phosphorus trichloride may have potential application for polypeptides synthesis.

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