Oligomerization of *N*,*O*-Bis(trimethylsilyl)-α-amino Acids into Peptides Mediated by *o*-Phenylene Phosphorochloridate

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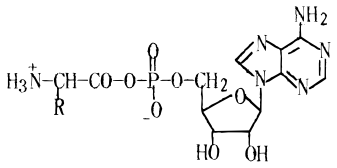
Received February 27, 1998

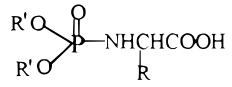
Abstract: *N*,*O*-Bis(trimethylsilyl)-α-amino acids (*N*,*O*-BTMS-AA), mediated by *o*-phenylene phosphorochloridate (PPC), could oligomerize into polypeptides. The mechanism might go through sequential steps, i.e., the activation of amino acid, the elongation of peptide chain, and the termination of elongation reaction, as can be traced by ^{31}P NMR spectroscopy. The activated amino acid was a five-membered cyclic pentacoordinate phosphoric−carboxylic mixed anhydride. The nucleophilic attack of the amino group of an amino acid or a peptide on the carbonyl group of the intermediate led to the formation of peptide with release of a phosphate ester. The repetition of the reaction sequence generated successively longer *N*,*O*-bis(trimethylsilyl)peptides, which were then hydrolyzed to give a series of oligopeptides. It is worth noting that only the *N*,*O*-bis-(trimethylsilyl)-α-amino acids, not the *N*,*O*-bis(trimethylsilyl)-*β*-amino acids, could be activated and assemble into polypeptides. The mechanism of the five-membered cyclic pentacoordinate phosphoric-amino acid anhydride intermediate showed that phosphorus could choose α-amino acids in the prebiotic synthesis of polypeptides and biosynthesis of proteins.

Introduction

The prebiotic synthesis of biopolymers probably involved the condensation and dehydration of active monomers under the conditions prevailing in the primeval ocean.[1] Some models have been proposed to explain the polycondensation process on peptide formation in aqueous media. Paecht-Horowitz et al. have studied the polymerization of free and substituted amino acid phospho-anhydrides[2−5] which are energy-rich monomers to permit the formation of peptide bonds even at high dilution and physiological PH and at room temperature. The most interesting of these experiments should be the prebiotic synthesis of polypeptides by heterogeneous polycondensation of amino acid adenylates (**I**). On the other hand, the formation of peptide and protein from amino acids is a central biosynthetic process in all living systems. Each amino acid is chemically activated, overcoming the thermodynamic barrier and kinetic pathway by formation of an aminoacyl adenylate. The initial step in the process catalyzed by the synthetase is activation of the specified amino acid by reaction with ATP to form the aminoacyl adenylate. The aminoacyl adenylates are enzymic reaction intermediates, reacting on the same enzyme with appropriate RNA to form an aminoacyl tRNA.[6] However, why natural proteins usually contain only α-amino acid residues on the backbone of peptide chain has not been explained. In our research group, *N*-phospho-α-amino acids have been studied in a water/alcohol system under mild conditions. Some interesting biochemical reactions were observed, e.g., *N*-phospho-α-amino acids could self-activated to give *N*-phosphoryl peptides,[7] *N*-phosphoamino acid esters,[8] ester-exchanged products on phosphorus,[9] and an intramolecular phosphoryl group migration from nitrogen to oxygen.[10] These results showed that the origin of life might be attributed to the evolution of *N*-phosphoamino acids.[11,12] All the above experiments were carried out in aqueous



Aminoacyl adenylate (I)



N-phospho--α-amino acid (II)

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10.1021/ja980662c CCC: $18.00
Published on Web 01/05/1999

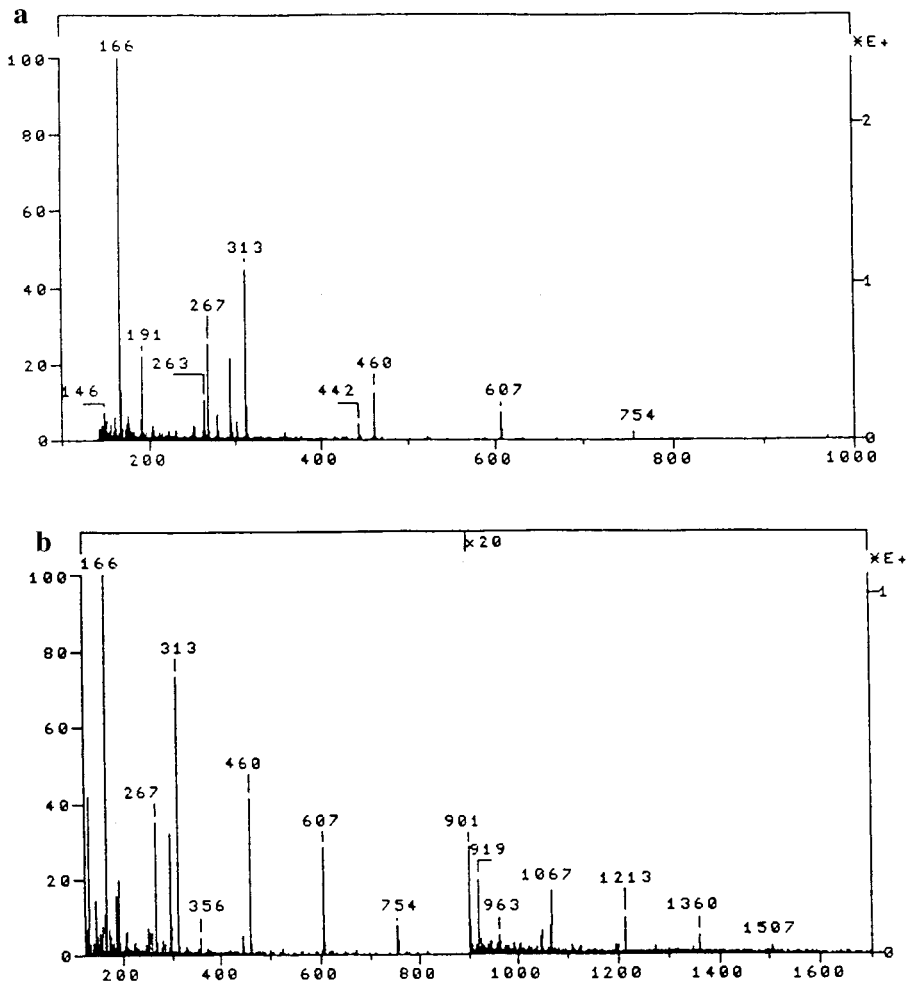

Figure 1. (a) FAB mass spectrum of oligomers for *N*,*O*-BTMS-Phe mediated by PPC for 12 h. (b) FAB mass spectrum of oligomers for *N*,*O*-BTMS-Phe mediated by PPC for 72 h.

media, and the activated amino acids could be observed in the forms of zwitterion (**I**) or *N*-phosphoamino acids (**II**)—their further reaction intermediates were not found. We would like to report herein that the *N*,*O*-bis(trimethylsilyl)-α-amino acid, mediated by PPC, could oligomerize into polypeptides in the aprotic solvents to avoid the affect of water on amino acid phospho-anhydrides; the five-membered cyclic pentacoordiate phosphoric-amino acid anhydride intermediates were observed. The phosphoryl group acted as the activating reagent, then left the backbone after peptide formation. It is worth noting that *N*,*O*-bis(trimethylsilyl)-*β*-amino acids did not give the same result.

Experimental Section

Preparation of Oligopeptides. General Procedure. To a stirred solution of *o*-phenylene phosphorochloridate[13] in anhydrous benzene (10 mL) at room temperature under a nitrogen atmosphere was added an excess of *N*,*O*-bis(trimethylsilyl)amino acids[14] (0.75 mmol); the reaction lasted 12−72 h. The solvent was removed by rotary evaporation, and the residue was dissolved in 0.1 N HCl (10 mL) solution. Some byproducts were extracted with ethyl acetate (3 × 5 mL). The oily crude product was obtained after lyophilization of the remaining solution, which was determined by reversed-phase HPLC, positive ion FABMS, and ^{1}H NMR spectroscopy.

Chromatography. The reaction product of *N*,*O*-BTMS-Phe with PPC was analyzed and prepared by reversed-phase HPLC. Sample analysis was performed on a SHIMADZU chromatograph, model LC-9A, with a SHIMADZU SPD-6AV detector. A ZORBOX ODS reversed-phase C18 column, 4.6 mm × 15 cm, was used. Sample preparation was carried out on a Waters 2010 HPLC with a 486 variable wavelength UV detector. A ZORBAX ODS semipreparative C18 column, 0.94 mm × 25 cm, was used. Elution was accomplished with a linear gradient of 100% A−30% B [(A) water, 0.1% TFA; (B) 95% acetonitrile−water, 0.05% TFA] over 30 min at a flow rate of 1.0 mL/min for analysis of the sample and 4.0 mL/min for preparation with a UV detector at 254 nm.

Analysis of Mass Spectrometry and NMR. Positive ion FAB mass spectra were recorded with a Finnigan MAT 90 double-focusing instrument (Finnigan MAT, Bremen, Germany) of BE geometry. The ion gun was operated at 5 mA current and 20 keV energy with Cs^+ ion as a bombarding ion. Glycerol was used as the matrix. The scan rate was 5 s/decade, and resolution of the instrument in the double-focusing model was adjusted to 10000 (10% valley definition). ^{1}H, ^{1}H−^{1}H COSY, and ^{1}H−^{1}H NOESY NMR spectra were recorded on a Bruker AM 500 spectrometer equipped with an ASPECT-3000 computer in DMSO-d_6 solvent with chemical shifts referenced to DMSO-d_6 (δ_H = 2.49 ppm). ^{31}P NMR spectra were performed on a Bruker AC 200p spectrometer in dry benzene solvent with use of 85% H_3PO_4 (δ_P = 0 ppm) as an external standard.

Results and Discussion

Amino acids are zwitterions and are not soluble in aprotic organic solvents. However, their trimethylsilyl derivatives, *N*,*O*-bis(trimethylsilyl)amino acids, are very soluble in organic solvents such as benzene and dichloromethane. *N*,*O*-Bis(trimethylsilyl)amino acids were prepared by refluxing the

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Table 1. FAB Mass Spectral Data of Oligomers for *N,O*-BTMS-Phe Mediated by PPC for 72 h (relative intensity in parentheses)

N,O-Bis-AA	Gly	Ala	Val	Leu	iso-Leu	Phe
momomer	76 (100)	90 (100)	118 (100)	132 (40)	132 (51)	166 (100)
dimer	133 (58)	161 (64)	217 (70)	245 (100)	245 (100)	313 (74)
trimer	190 (37)	303 (35)	316 (46)	358 (18)	358 (22)	460 (40)
tetramer	247 (25)	374 (21)	415 (33)	471 (12)	471 (13)	607 (30)
pentamer	304 (16)	445 (14)	514 (12)	584 (8)	584 (10)	754 (8)
hexamer	361 (7)	516 (8)	613 (4.3)	697 (3.8)	697 (4)	901 (1.6)
heptamer	418 (2.1)	587 (1.4)	712 (1.2)	810 (1.8)	810 (1.5)	1048 (0.4)
octamer	475 (0.5)	658 (0.8)		923 (0.3)	923 (0.5)	1195 (0.2)

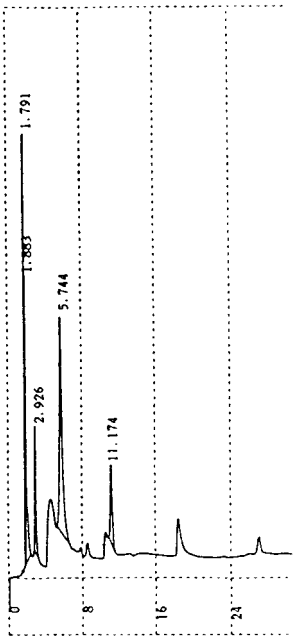


Figure 2. HPLC elution profile of oligomers for *N,O*-BTMS-Phe mediated by PPC for 12 h.

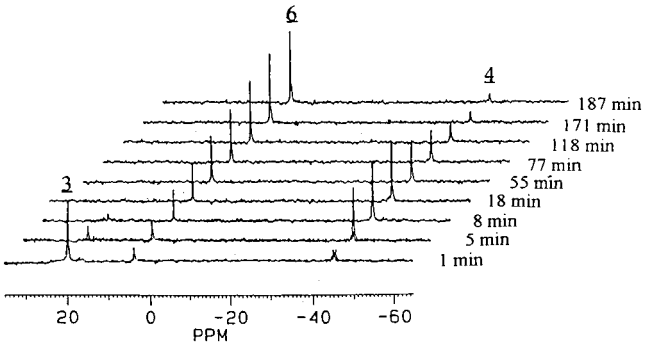


Figure 3. The stack ^{31}P NMR spectra of *N,O*-BTMS-Phe oligomerization into peptides mediated by PPC in dry benzene solvent.

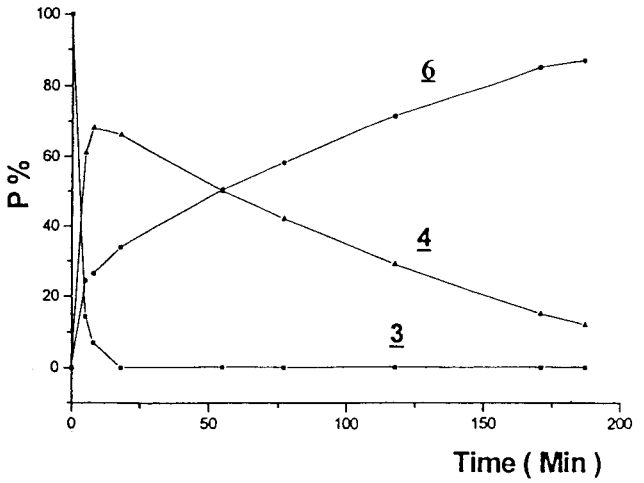


Figure 4. The change curves of the relative intensity of components containing phosphorus corresponding to Figure 3 with times.

corresponding amino acids with hexamethyldisilazane according to the reported procedure,[13] and they show some similar chemical specificity to natural amino acids for easy migration of the trimethylsilyl group as the proton.

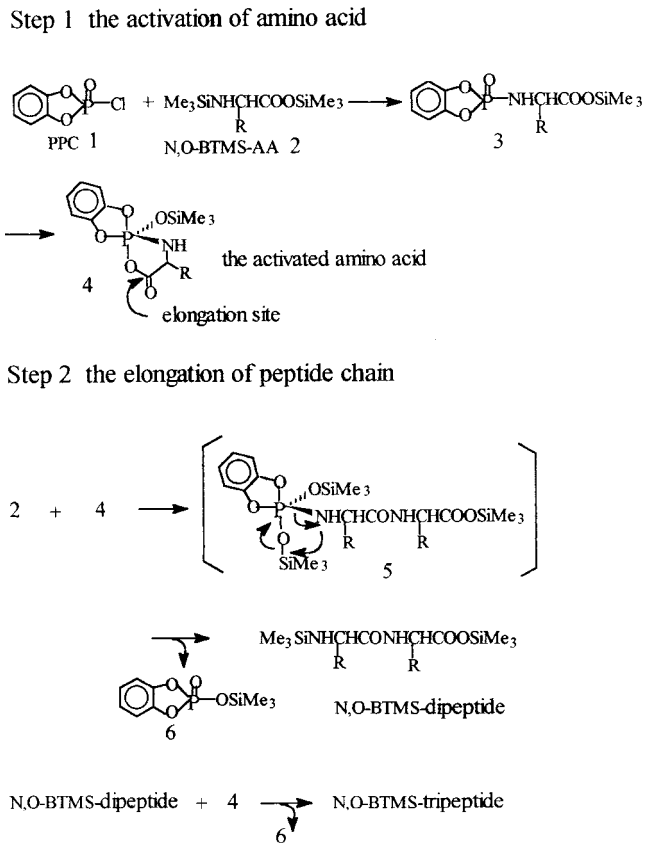
1. Analysis of the Oligomers. The oligomers resulting from the reaction of *N,O*-bis(trimethylsilyl)phenylalanine and PPC for 12 h are chosen as a typical example. Their positive ion FAB mass spectrum is shown in Figure 1a. A series of mass numbers (166 + 147*n*) were found, which corresponded to oligopeptides from monomer to pentamer of phenylalanine. Correspondently, the HPLC at UV 254 nm showed five peaks, at 1.79 (100%), 5.74 (42%), 11.17 (14%), 18.42 (8%), and 27.23 (3%) min (relative amount in parentheses), as shown in Figure 2. Both results seem to be in good agreement with peak numbers of oligopeptides and the relative amount of each component. The five main components in Figure 1b were prepared by reversed-phase semipreparative HPLC. Tripeptide ^{1}Phe^{2}Phe^{3}-Phe corresponding to the peak at 11.17 min in Figure 2 was characterized by ^{1}H, ^{1}H−^{1}H COSY, and ^{1}H−^{1}H NOESY NMR, and its ^{1}H NMR chemical shifts are as follows: ^{1}Phe 2.87, 3.12 (C$^{\beta}$H), 3.96 (C$^{\alpha}$H), 8.10 (NH); ^{2}Phe 2.80, 2.99 (C$^{\beta}$H), 4.57 (C$^{\alpha}$H), 8.92 (NH); ^{3}Phe 2.93, 3.08 (C$^{\beta}$H), 4.47 (C$^{\alpha}$H), 8.58 (NH).

We found that as the reaction time was prolonged, not only the amounts of peptide increased but also the length of the determined peptides increased. For example, Figure 1b shows the positive ion FAB mass spectrum of the reaction product of *N,O*-BTMS-AA with PPC for 72 h. Compared with Figure 1a, the relative intensity of dipeptide PhePhe increased from 44% in Figure 1b to 72% in Figure 1b, and correspondently, the longest peptide rose from pentamer to octamer.

Oligopeptides formed for other *N,O*-bis(trimethylsilyl)amino acids in 72 h were also determined by positive ion FABMS, and the results are depicted in Table 1. The protonated molecular ions corresponding to oligopeptides from monomer to octamer were observed.

2. Mechanism of *N,O*-BTMS-AA Mediated Oligomerization into Peptides by PPC. The FABMS results described previously showed that the length of the peptides was dependent on reaction times. *N,O*-BTMS-AA could hardly form any peptide in the absence of PPC at room temperature. This implied that PPC must mediate these reactions. ^{31}P NMR spectroscopy is the most important tool for monitoring the reaction mechanistic course of phosphorus compounds, and it can be used to investigate the chemical and stereochemical behavior of reaction intermediates, whose isolation is difficult or even impossible. For example, under a nitrogen atmosphere, 0.1 mmol of PPC and 0.15 mmol of freshly prepared *N,O*-BTMS-AA were added

Scheme 1. The Possible Mechanism of Self-Assembly into Peptide for N,O-BTMS-AA Mediated by PPC



to 0.6 mL of dry benzene in an NMR tube. The progress of the reaction was monitored by ^{31}P NMR spectroscopy as shown in Figure 3.

After 1 min, the starting material **1** (δ_P = 19 ppm) was quickly translated into **3** at ^{31}P NMR 19.8 ppm, then **3** was completely converted into **4** (δ_P = −45.4 ppm) within 8 min along with the appearance of **5** at ^{31}P NMR 3.5 ppm.[15] As the reaction continued, the amount of **4** gradually decreased, while the amount of **6** increased as shown in Figure 4.

^{31}P NMR stack results seem to indicate a possible mechanism as shown in Scheme 1. **Step 1** is the activation of amino acid—the formation of pentacoordiate phosphoric-carboxyl mixed anhydride. The reaction of *N,O*-BTMS-AA (**2**) with PPC (**1**) led to *N*-(*o*-phenylene)phosphoamino acid trimethylsilyl ester (**3**), then **3** isomerized into a pentacoordinate phosphoric-amino acid mixed anhydride **4**, whose structure was proved by an authentic sample.[16] Anhydride **4** was considered to be the activated amino acid. **Step 2** is the elongation of the peptide chain. Nucleophilic attack of amino acid or peptide trimethylsilyl derivativers led to a series of oligopeptides. Nucleophilic attack of the secondary amino group of another *N,O*-BTMS-AA on the carbonyl of **4** formed **5** containing an amide bond. **5** changed into *N,O*-BTMS-dipeptide (**7**) leaving a phosphotriester (**6**). Repetition of this sequence of reactions generated successively longer peptides. **Step 3** is the termination of the elongation reaction. When the activated amino acids were used up, the oligomerization reactions stopped. The degree of peptide elongation was dependent on the availability of the activated amino acids. Among the three steps, the activation of the amino acid is the key step.A control experiment was performed with the same conditions. Consequently, reaction of *N,O*-bis(trimethylsilyl)-β-alanine with PPC only produced *N*-(*o*-phenylene)-β-alanine trimethylsilyl ester at ^{31}P NMR 20.8 ppm. It is interesting to note that the pentacoordinate phosphoric-carboxylic mixed anhydride was not observed with the ^{31}P NMR method. Positive ion FABMS of the resulting solution after hydrolysis of 0.1 N HCl only showed the protonated molecular ion of β-alanine at *m/z* 90; no peptide, however, was found. This might be explained in light of the knowledge of phosphorus chemistry, in which a five-membered cyclic phosphorane is energetically much more favorable than a six-membered cyclic one. The pentacoordinate phosphoric-carboxylic mixed anhydrides formed by α-amino acids have a five-membered ring, however with β-type acids, a six-membered ring is formed. The former is more readily formed and stable than the latter.

These results implied that there might be an equilibrium existing in the biosynthesis of protein as shown in Scheme 3. The five-membered cyclic pentacoordinate phosphoric-amino acid mixed anhydride (**7**) similar to **4** in Scheme 1 might be an important intermediate of the aminoacyl-AMP **9**. The biologically relevant phosphatidylethanolamine and phosphatidylserine on headgroup cyclization were also suggested to yield five-membered cyclic phosphoramidate intermediates.[17,18] So phosphorus might choose α-amino acids when the aminoacyl-AMP is formed in the biosynthesis of protein, this α-amino acid specificity might disclose why there only are α-amino acids on the backbones of natural proteins.

Conclusion

The mechanism that only *N,O*-bis(trimethylsilyl)-α-amino acid, mediated by *o*-phenylene phosphochloridate, could form five-membered cyclic phosphoric-amino acid anhydride and assemble into peptides seems to suggest that phosphorus plays

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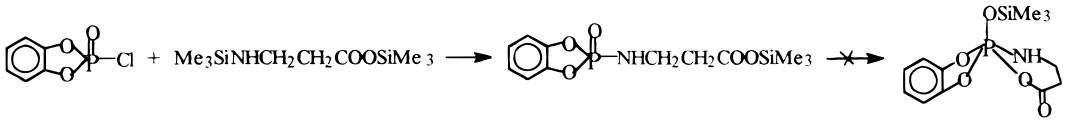
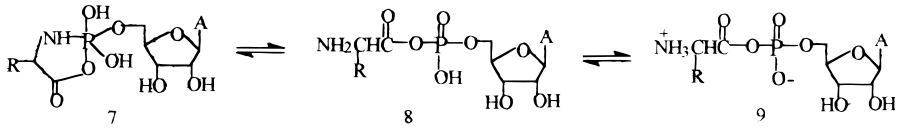
Scheme 2. Reaction of *N,O*−BTMS-*b*-alanine with PPC



Scheme 3. The Possible Five-Membered Cyclic Intermediate of Aminoacyl-AMP in the Biosynthesis of Protein



important roles in the prebiotic synthesis of polypeptide and biosynthesis of protein; it could be phosphorus that chooses α-amino acid depending on its five-membered cyclic specificity. On the other hand, the pentacoordinate phosphoric-amino acid anhydride formed by an equiomolar amount of *o*-phenylene phosphochloridate and *N,O*-bis(trimethylsilyl)-α-amino acid in dilute aprotic solution could exist in the stable form for 2−6 h—organic phosphorus protects the amino group of amino acid and activates its carboxyl and the activated amino acids have one potential application for polypeptide synthesis like *N*-carboxy-anhydrides.[19−22]

Acknowledgment. The authors wish to acknowledge the financial support from the National Natural Science Foundation of China, the National Science and Technology Committee of China, Chinese National Ministry of Education and Tsinghua University.

JA980662C