Ligand Exchange Between Penta-Coordinated Phosphoryl Serine and Histidine Compounds

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With the assistance of HPLC-ESI-MS/MS, the self-assembly products of serine and histidine penta-coordinated phosphorus compound were separated and identified. The expectative product was seryl-histidine dipeptide, but it was found that there was almost equimolar amount of histidyl-histidine dipeptide as well as seryl-histidine dipeptide. The mechanism was speculated that there was ligand exchange between penta-coordinated phosphoryl serine and histidine in the reaction process. As a result, two types of dipeptide were produced.

Keywords seryl-histidine dipeptide, histidyl-histidine dipeptide, penta-coordinated phosphorus compound, HPLC-ESI-MS/MS

The penta-coordinated phosphorus compounds play a special and important role in phosphorus chemistry. For example, the reactions of many tri-, tetra-coordinated phosphorus compounds went through penta-coordinated phosphorus intermediates.[1,2] Penta-coordinated phosphorus compounds are very important in biochemistry. Many intermediates of phosphate ester compounds as enzyme substrate were proposed as penta-coordinated phosphorus structure.[3] Penta-coordinated phosphorus compounds have been a very active field in the past forty years.[4,5]

Recently, it was found that *N*-phospho-α-amino acids could induce many kinds of bioorganic reactions in water-alcohol media,[6-8] such as peptide formation, ester formation, ester exchange and N→O phosphoryl migration. These reactions underwent the penta-coordinated phosphorus amino acid intermediates, which were unstable in the water-alcohol system and could not be easily detected. In order to confirm the penta-coordinated phosphorus intermediates, an interesting experiment involving silicon chemistry was applied to trap the penta-coordinated phosphorus intermediates.[9,10] It was found that *N*,*O*-bis(trimethylsilyl)-α-amino acids could be mediated by *O*,*O*-phenylene phosphorochloridate to oligomerize into polypeptides.[2] The mechanism went through penta-coordinated phosphorus amino acid intermediates. For the synthesis of seryl-histidine dipeptide (Ser-His), the first example of metal-ion-free hydrolytic DNA cleavage agent,[11,12] *N*,*O*-bis(trimethylsilyl) serine trimethylsilyl ester was phosphorylated by *O*,*O*-phenylene phosphorochloridate, then the penta-coordinated phosphorus serine reacted with *N*,*N*-bis(trimethylsilyl) histidine trimethylsilyl ester to synthesize Ser-His. In this process, HPLC-ESI-MS/MS was used to separate and identify the reaction products. It was found that there was almost equimolar amount of histidyl-histidine dipeptide (His-His) as well as Ser-His in the synthesized products. The formation mechanism of His-His was discussed.

Experimental

Instruments

^{31}P NMR spectra were recorded on a Bruker DPX400MHz spectrometer in $CDCl_3$ with 85% H_3PO_4 as the external standard. HPLC-ESI-MS/MS was obtained on a Bruker Esquire 3000 LC-MS spectrometer with positive model.

The HPLC conditions are as follows. An HP1100 Series HPLC instrument was used, consisting of a degasser, binary pump, manual injector and a UV detector. Detection was done by UV absorbance at a wavelength of 210 nm and a bandwidth of 2 nm. Reversed-phase HPLC was performed on a column (Microsorb C18 4.6 mm × 150 mm, 5 μm). Analyses were performed at a flow-rate of 0.5 mL/min. The mobile phase was a mixture of acetonitrile and water (30:70, *V*/*V*) and 1 mmol/L sodium dodecyl sulphonate (SDS) and 0.05% trifluoroacetate (TFA) were added to water.

Mass spectra were acquired on a Bruker Esquire 3000

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ion trap mass spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m/z* 6000. Nitrogen was used as drying gas with the flowing rate of 4 L/min. The nebulizer is 7 psi. Capillary was typically held at 4 kV. The samples dissolved in methanol and water were ionized by electrospray ionization and continuously infused into the ESI chamber at a flow rate of 4 μL/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). The selected ions from the mixture were analyzed by tandem mass spectrometry.

Reagent

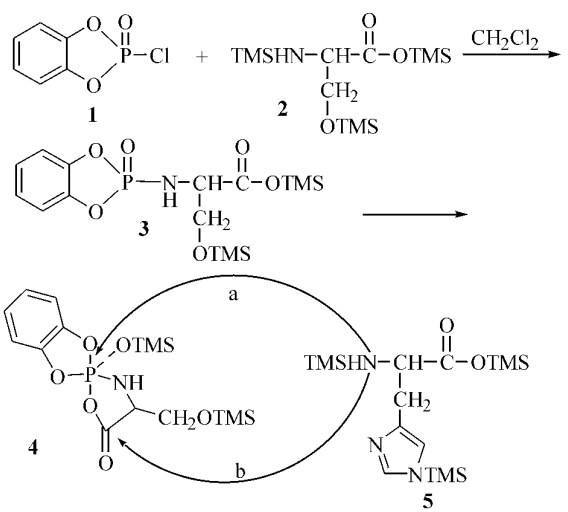
O,*O*-Phenylene phosphorylchloride (**1**),[13] *N*,*O*-bis-(trimethylsilyl) serine trimethylsilyl ester (**2**)[14] and *N*,*N*-bis(trimethylsilyl) histidine trimethylsilyl ester (**5**)[14] were obtained according to the literature. All chemicals were of chemical grade or analytical grade. Toluene and dichloromethane were dried and distilled. The apparatus were dried fully. All reactions were carried out under a highly pure nitrogen atmosphere.

Experimental procedure

Synthesis of penta-coordinated phosphoryl serine (**4**)

A solution of **2** (6.41 g, 20.0 mmol) in anhydrous CH_2Cl_2 (40 mL) was added dropwise to the solution of **1** (3.82 g, 20.0 mmol) in anhydrous CH_2Cl_2 (60 mL) at −50— −60 ℃. Then, the reaction mixture was stirred at −30—40 ℃, and monitored by ^{31}P NMR spectra until the reaction was completed by the diminish of reactant **3**. Compound **3** was isomerized into a pepta-coordinated phosphoric amino acid mixed anhydride **4**, whose structure was proved by an authentic sample.[2,9,15] The synthesis of the serine penta-coordinated phosphorus **4** is shown in Scheme 1.

Scheme 1 Synthesis of serine penta-coordinated phosphorus (**4**)



Synthesis of dipeptide by **4** *and* **5**

A solution of freshly prepared **5** (7.42 g, 20.0 mmol) in anhydrous CH_2Cl_2 (40 mL) was added dropwise to the solution of **4** (20.0 mmol) in anhydrous CH_2Cl_2 at −30—40 ℃. The reaction mixture was stirred at room temperature, and monitored by ^{31}P NMR spectra until the peak of **4** disappeared. After solvent was removed by a rotary evaporator, the residue was hydrolyzed with 50 mL of water in an ice-water bath. The mixture was adjusted to pH = 6 with dilute hydrochloric acid. The solution was stirred for 1 h at room temperature, and fully extracted with ethyl acetate. The water layer was freeze-dried to afford a pale brown solid, which was analyzed by HPLC-ESI-MS/MS.

Results and discussion

Product identification

In all mass spectra of products, the peaks at *m/z* 156, 243 and 293 correspond to $[His + H]^+$, $[Ser\text{-}His + H]^+$ and $[His\text{-}His + H]^+$ ions, respectively. From the results it seemed that there were His-His in addition to Ser-His in reaction mixture. There are many methods, such as ion exchange chromatography and reversed-phase high performance liquid chromatography[16] for separation of amino acid and oligopeptide in recent years. By reversed-phase ion pair HPLC-ESI-MS/MS, the Ser-His and His-His were separated and quantified. The LC chromatography is shown in Fig. 1. The peak eluted at 10.6 min, whose *m/z* was at 243, is interpreted as protonated Ser-His. Of course, the peak eluted at 23.0 min, whose *m/z* was at 293, is interpreted as protonated His-His. The contents of dipeptides were calculated by area percent method. The concentration of Ser-His was about 15% and the concentration of the His-His was about 14%.

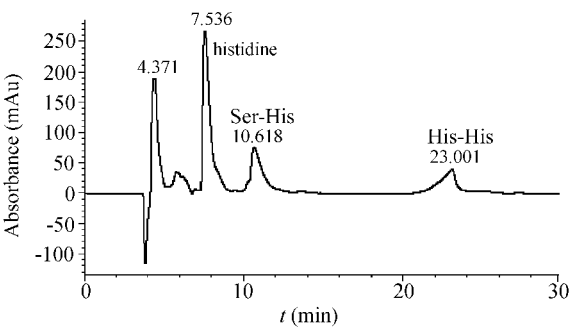
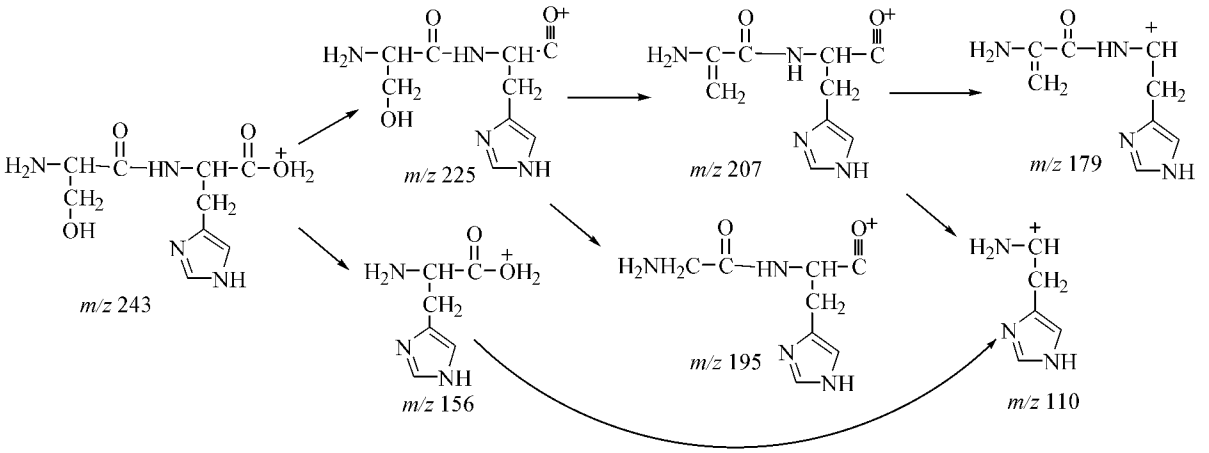


Fig. 1 LC-UV spectrum of reaction mixture.

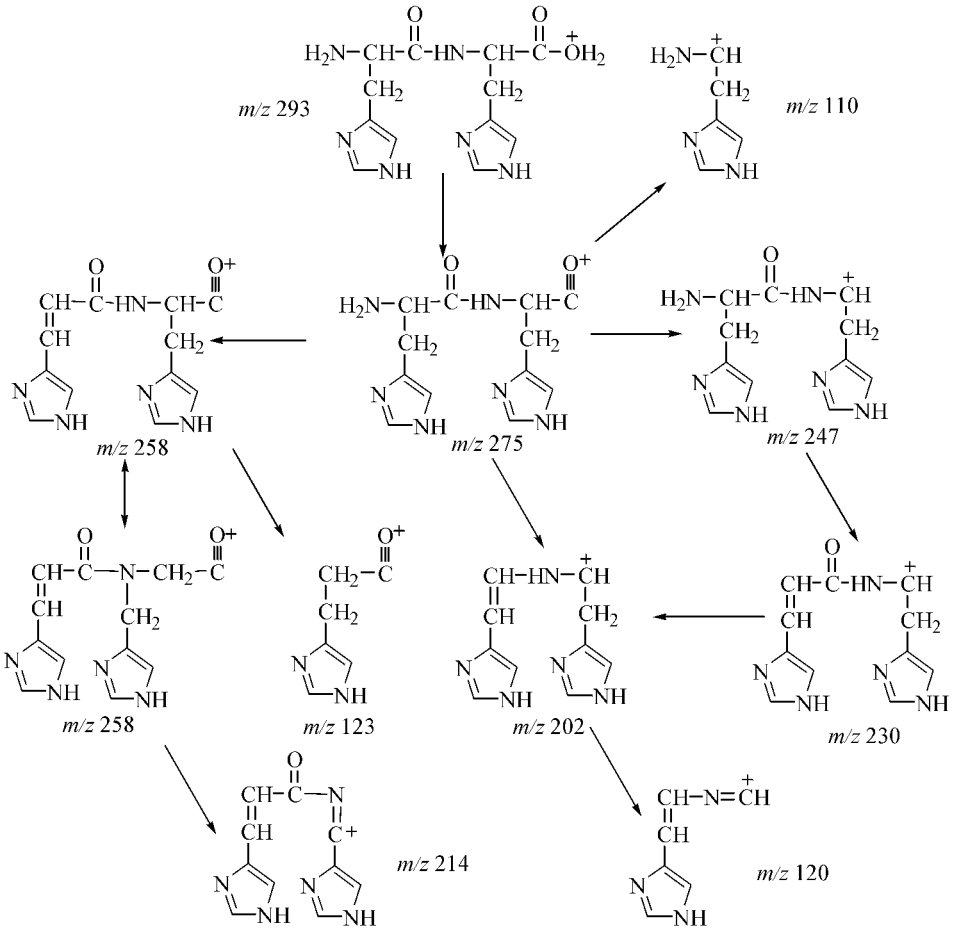
The MS/MS spectral data of the *m/z* 243 and *m/z* 293 ions, and of the most significant fragment ions are summarized in Table 1.

On the basis of HPLC-ESI-MS/MS, the ion at *m/z* 243 was interpreted as Ser-His, and the ion at *m/z* 293 was identified as His-His. Fragmentation pathways of dipeptides are shown in Schemes 2 and 3.

Scheme 2 Fragmentation pathway of Ser-His



Scheme 3 Fragmentation pathway of His-His



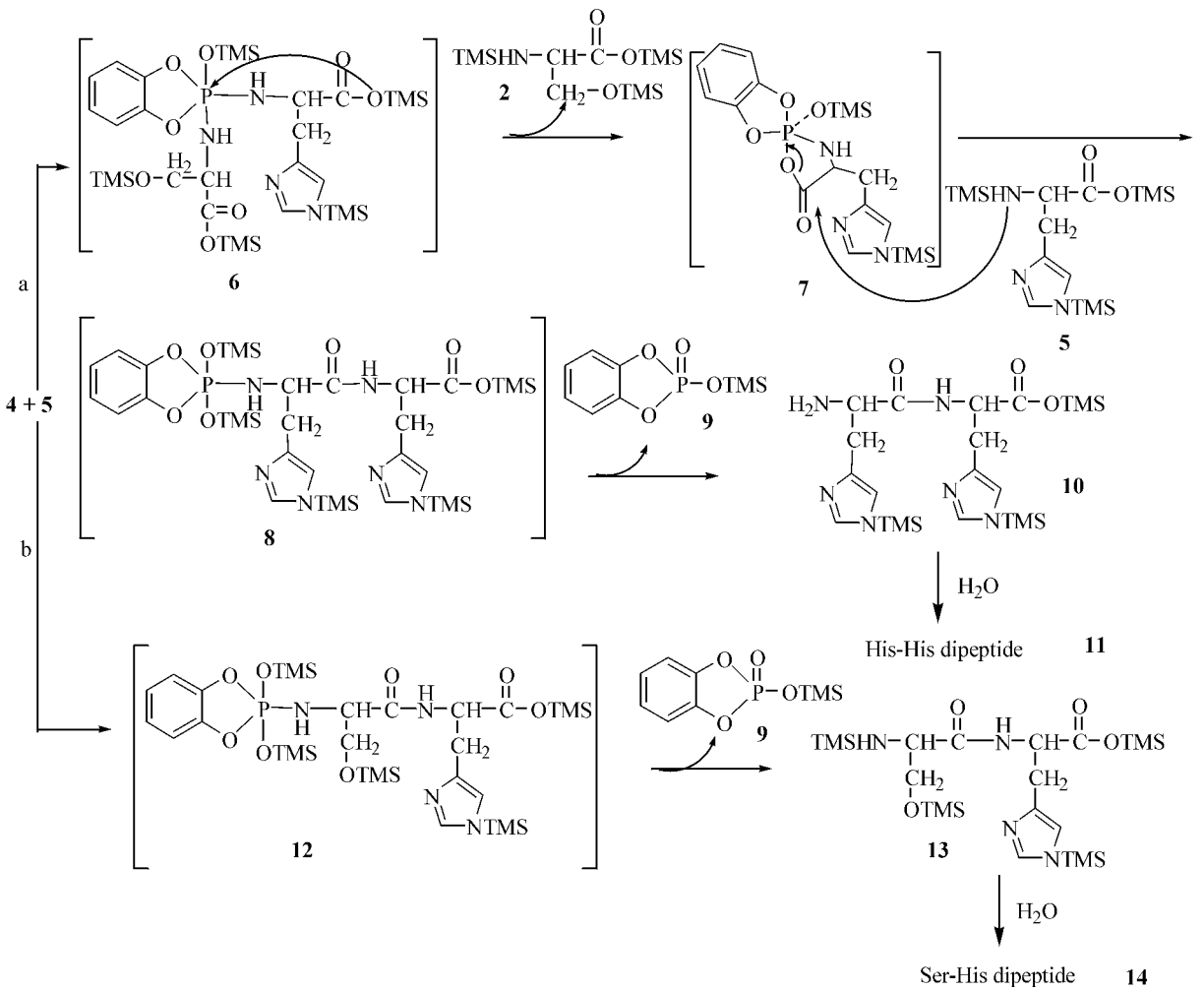
Proposed competition mechanism

Serine penta-coordinated phosphorus **(4)** was obtained as shown in Scheme 1. The self-assembly reaction of **4** was traced by ^{31}P NMR. It was found that if the reaction temperature was high, there were signals at δ −34.7 and δ −35.9 as the isomerization product of **4** in addition to two signals at δ −44.0 and δ −44.4. Isomers were formed by the nucleophilic attack of the hydroxyl group of serine side chain.[15] The isomerization could be reduced by controlling the reaction temperature. Moreover, from the literature,[9] it was supposed that when penta-coordinated phosphorus compound was formed, the formation rate constant of histidine was 2.53 times faster than that of serine because of the catalysis of imidazole ring. Therefore, two likely pathways leading to two penta-coordinated phosphorus compound might happen in Scheme 1. Because the basicity of protected imidazole nitrogen was weaker than the

Table 1 Positive ion ESI-MS/MS data for dipeptide

Compound	Precursor ion (m/z)	Main fragment ions [m/z, %)]
Ser-His	243[M + H]$^+$	243 (48), 225 (100), 195 (4), 156 (7)
	225	225 (5), 207 (89), 195 (100), 110 (73)
	207	207 (26), 179 (100), 110 (20)
His-His	293 [M + H]$^+$	293 (28), 275 (100)
	275	275 (18), 258 (100), 247 (13), 214 (22), 202 (70), 110 (35)
	258	258 (21), 214 (100), 123 (11)
	202	202 (33), 120 (100)
	247	247 (47), 230 (18), 202 (100)
	230	230 (6), 202 (100)

Scheme 4 Proposed mechanism for the His-His formation



protected amino nitrogen, the steps of (a) and (b) were the main nucleophilic attack pathways. It was suspected that the formation of His-His might go through the histidine penta-coordinated phosphorus (**7**) which was formed by the subsitution of *N*,*N*-bis (trimethylsilyl) histidine trimethylsilyl ester (**5**) for *N*,*N*-bis (trimethylsilyl) serine trimethylsilyl ester (**2**) as shown in Scheme 4. Then compound **7** was attacked by the nitrogen atom of **5** to give the His-His after hydrolysis.

Accordingly, there was a competition reaction between the formation of **4** and **7**. They could be attacked by *N*,*N*-bis(trimethylsilyl)amino acid trimethylsilyl ester. For the purpose of verifying the mechanism, the seryl-phenylalanine dipeptide and the seryl-asparagine dipeptide have been synthesized by this method. Similarly, in the reaction mixture there were competition products such as phenylalanyl-phenylalanine dipeptide or aspartyl-asparagine dipeptide that were identified by the ESI-MS/

MS, respectively. Therefore, it was proposed that there was the ligand exchange of penta-coordinated phosphorus compound in the amino acid self-assembly reaction by the action of the phosphorous reagent.

Conclusion

With the assistance of HPLC-ESI-MS/MS the products from the reaction of *N*,*N*-bis(trimethylsilyl) histidine trimethylsilyl ester with penta-coordinated phosphoryl serine were separated and identified. It was found that there were almost equal amount of His-His and Ser-His, and was proposed that the ligand exchange between penta-coordinated phosphoryl serine and histidine could happen easily. This method to synthesize dipeptide can be improved to get reasonable fields through further study.

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