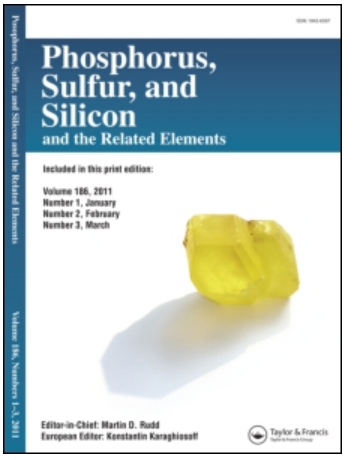

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PHOSPHORYL PROMOTION AND DIFFERENTIATION EFFECT ON AMINO ACIDS AND PREBIOTIC SYNTHESIS OF PROTEIN

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PHOSPHORYL PROMOTION AND DIFFERENTIATION EFFECT ON AMINO ACIDS AND PREBIOTIC SYNTHESIS OF PROTEIN

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In the presence of phosphoryl group, N-phosphoamino acids become more reactive and self-catalyzed to form peptides by coupling with amino acids or their esters at low temperature in different solvents, even water. It might be important for the prebiotic synthesis of a protein.

Key words: N-phosphoamino acid, self-catalysis, mixed anhydride intermediate, prebiotic synthesis.

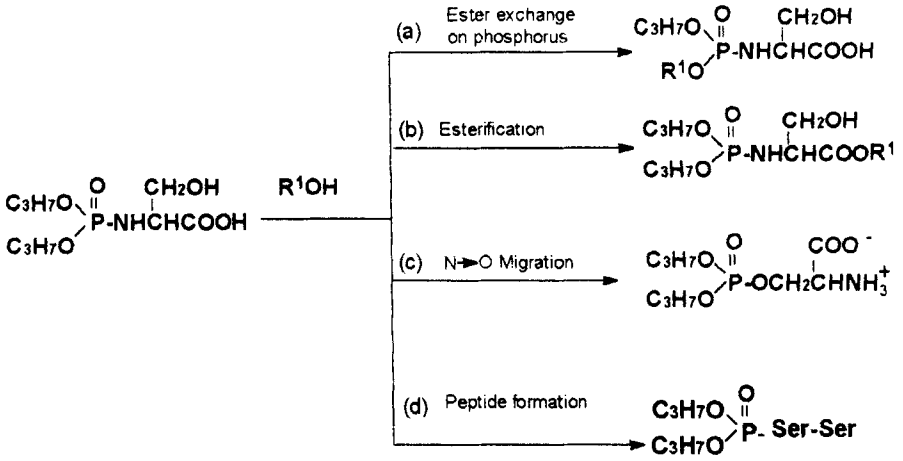
Phosphorus and amino acids play important roles in life chemistry. Although many reports on peptide formation reactions in the process of chemical evolution have been published,[1-4] the proteins formed on the primitive earth are still under discussion and there is not much investigation on the intrinsic relationship between the phosphorus and amino acids. In our laboratory, a number of N-phosphoryl amino acids and peptides have been synthesized and it was found that dialkyl phosphite is a new coupling reagent for peptide synthesis.[5-8] Here, we wish to report that N-phosphoamino acids, because of the presence of the phosphoryl group, become more reactive and can self-catalyze to form the peptide at low temperature in different solvents, even in an aqueous medium.

RESULTS AND DISCUSSION

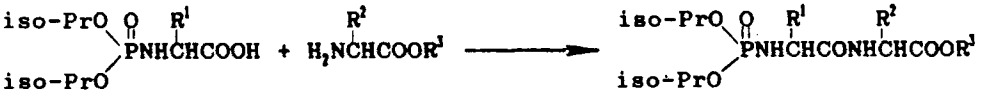
In order to understand the chemistry of the phosphoprotein, the amino acid residues were phosphorylated and their chemical properties were investigated.[6-8] It seems that the existence of a phosphoryl group leads to many interesting chemical properties. For example, the phosphorylated serine tends to self-activate to give peptides, esters, phosphoryl esterification and N → O migration of the phosphoryl group (Scheme I). In which, the phosphoryl participation reactions are similar to the foundations of bio-catalysis.[8-11]

Phosphoryl Group Promotion Effect

In general, biologic reactions take place in aqueous media, therefore, the reactions of N-phosphoamino acids in aqueous solution were studied at 38°C. It was found that N-phosphoamino acids can form homopeptides and heteropeptides as coupled by other amino acids or amino acid esters (Scheme II).



SCHEME I Chemical properties of N-phosphoryl serine.



SCHEME II

In our previous paper,[12,13] it was shown that FAB mass spectrometry was a very useful method for determining the structure of phosphorylated peptides. By which means the possible peptides structure can be established on the basis of the molecular ion M^+ or pseudomolecular ion and the corresponding fragment ions M-42, M-42 × 2 in positive ion FAB-MS. For example, due to the presence of the phosphoryl group, a very intense molecular ion M^+ peak together with the fragments ions $[M-42]^+$ and $[M-2 \times 42]^+$ for the successive loss of one propene and two propene provided a triple check for formula weight identification. In addition, their IR spectra showing the amide band absorption at 1650 cm^{-1} further supported the peptide formation. The results are shown in Tables I–III.

However, the other types of N-protected amino acids, such as Boc-Pro, Z-Gly, Boc-His, Boc-Cys (where Boc = $(CH_3)_3OCO$—, Z = Ph—CH_2OCO—), could not form the peptide under similar condition, no peptide was observed by IR and FAB-MS.[5] It is also of note that the self-activation is a unique character of these N-phosphoamino acids. It seems that all of these reactions are attributed to the presence of the N-phosphoryl group.

Phosphoryl Group Differentiation Effect

It is known that histidine existed in the active site of many enzymes, such as chymotrypsin, pancreatic ribonuclease, and carboxyl peptidase, etc. and that phosphoryl histidine is present as an intermediate in reactions involving acid phosphatase, phosphofructokinase, and histamine kinase.[11] Thus, it is of interest to study the properties of phosphoryl histidine. The studies on phosphoryl transfer reactions

TABLE I

N-Phosphoryl histidine self-catalyzed to form peptides at 38°C in various different solvents

possible peptides	M⁺ and fragment ions in positive FAB-MS (m/z)			
	H_2O	Citrate Buffer pH=6.0	DMF	DMSO
DIPPHis	320(M+1)	320(M+1)	320(M+1)	320(M+1)
	419(M)	319(M)	319(M)	319(M)
DIPPHisHis	456(M,3.5%)	457(M+1)	457(M+1)	456(M)
	373(M-42x2+1)	415(M-42+1)	415(M-42+1)	415(M-42+1)
His-His	293(M+1)	292(M)	293(M+1)	293(M+1)
$DIPPHis(His)_2$	593(M,1%)	593(M)	594(M+1)	594(M+1)
	510(M-42x2+1)	510(M-42x2+1)	593(M)	510(M-42x2+1)
His-His-His		429(M)	430(M+1)	
$DIPPHis(His)_3$	648(M-42x2+1)	731(M)	731(M)	732(M+1)
		648(M-42x2+1)	648(M-42x2+1)	690(M-42+1)
				648(M-42x2+1)
$(His)_4$		586(M)	586(M)	586(M)

TABLE II

Self-activated peptide formation of some N-phosphoamino acids in water at 38°C

Reagent	possible peptide	M+ and fragment ions in positive FAB-MS (m/z)	M- and fragment ions in negative FAB-MS(m/z)
DIPPCys	DIPPCysCys	388(M, 1%),346(M-42, 1%)	388,346,304,342(M-46)
DIPPLys	DIPPLysLys	439(M+1, 0.3%)	438,396,354
DIPPAla	DIPPAlaAla	325(M+1, 1%),282(M-42, 8%)	323,324,279,236
DIPPGlu	DIPPGluGlu		440(M),398(M-42), 356(M-42x2)

of phosphoryl histidine have been reported.[7] Here, we successfully study the self-formation peptides from phosphoryl histidine.

In different media, the N-phosphohistidine can self-catalyze to elongate and form homopeptides or phosphoryl peptides (Table I). From Table I, the N-phospho-dipeptide and N-phosphotripeptides, even tetrapeptide, were found. It seems that there were about four kinds of histidine products derived from a single origin. The 200 MHz ^1H NMR spectra showed very broad imidazole proton peaks at the regions of 7.20–7.80 ppm and 8.2–8.4 ppm (Figure 1) contrary to the sharp peaks for pure histidine under similar condition. It indicated that there were several different histidine containing products. This conclusion could also be confirmed by the 500 MHz ^{13}C NMR of the crude products derived from DIPPHis. The ^{13}C NMR spectra gave overlapped peaks at the regions of 53.5–55.5 ppm corresponding to the α-carbons for different kinds of histidine in the crude products. Also, there were various imidazole sp^2 carbons at the regions 133.5–135.0, 129.0–131.0, 117.5–119.5 ppm. In addition, the carbonyl region gave five kinds of carbonyl signals at 163.3, 164.7, 167.2, 171.9 (broad), 172.9 ppm (Figure 1). It obviously suggested

TABLE III

Self-activated peptide formation of N-phosphohistidine with the amino acids and amino acid esters in water at 38°C

Reagent	possible peptide	M+ and fragment ions in positive FAB-MS (m/z)	M- and fragment ions in negative FAB-MS(m/z)
DIPPHis +Cys	DIPPHisCys	423(M+1, 1%) 381(M-42+1, 2%)	422(M),380(M-42), 348(M-42X2)
DIPPHis +CysOBu	DIPPHisCysOBu	478(M, 2%)	
DIPPHis +GlyGly	DIPPHisGlyGly	433(M, 1%),391(M-42, 2%)	432(M-1), 433(M),390(M-42-1)
	DIPPHisHisGlyGly	571(M, 1%)	
DIPPHis +GlyGlyOBu	DIPPHisGlyGlyOBu	490(M, 0.5%) 405(M-42x2, 1%)	489(M),447(M-42), 405
DIPPGlu +GlyGlyOBu	DIPPGluGlyGlyOBu	482(M+1, 0.2%), 440(M-42+1, 1%)	
DIPPAla +GlyGlyOBu	DIPPAlaGlyGlyOBu	424(M, 0.3%)	
DIPPCys +GlyGlyOBu	DIPPCysGlyGlyOBu	456(M+1, 0.2%), 371(M-42x2, 4.8%)	

that there were at least five different C=O groups. However, the pure histidine gives only one α—C, one set of imidazole sp^2-carbons and one C=O signal. Therefore, it was concluded that there were several peptides derived from the DIPPHis. The mechanism for the oligopeptide formation was proposed through the "1 + n" path (Scheme III), which was similar to the peptide formation mechanism in the r-RNA.

Table II shows that other kind of N-phosphoamino acids could also self-catalyze to form dipeptide.

Table III shows that N-phosphohistidine not only can activate itself to give the peptide, but also could couple with other amino acid esters to form the hetero-peptides.

As the results showed in Tables I–III, it indicated that N-phosphohistidine could not only give the dipeptide, but also the tripeptide, and even the tetrapeptide. But the other N-phosphoamino acid could only yield the dipeptides. The reason for the higher reactivity of phosphohistidine could be explained by the imidazole group intramolecular catalysis effect. Because of the imidazole group participation, the phosphorus might be transformed into a hexacoordinate intermediate (Scheme IV). The direct P—N bond from the participation of imidazole had also been proposed before.[7,14]

Lipmann[15] suggested that the bio-synthesis of protein proceeded through a mixed carboxylic-phosphoric anhydride intermediate, hence, based on these experimental

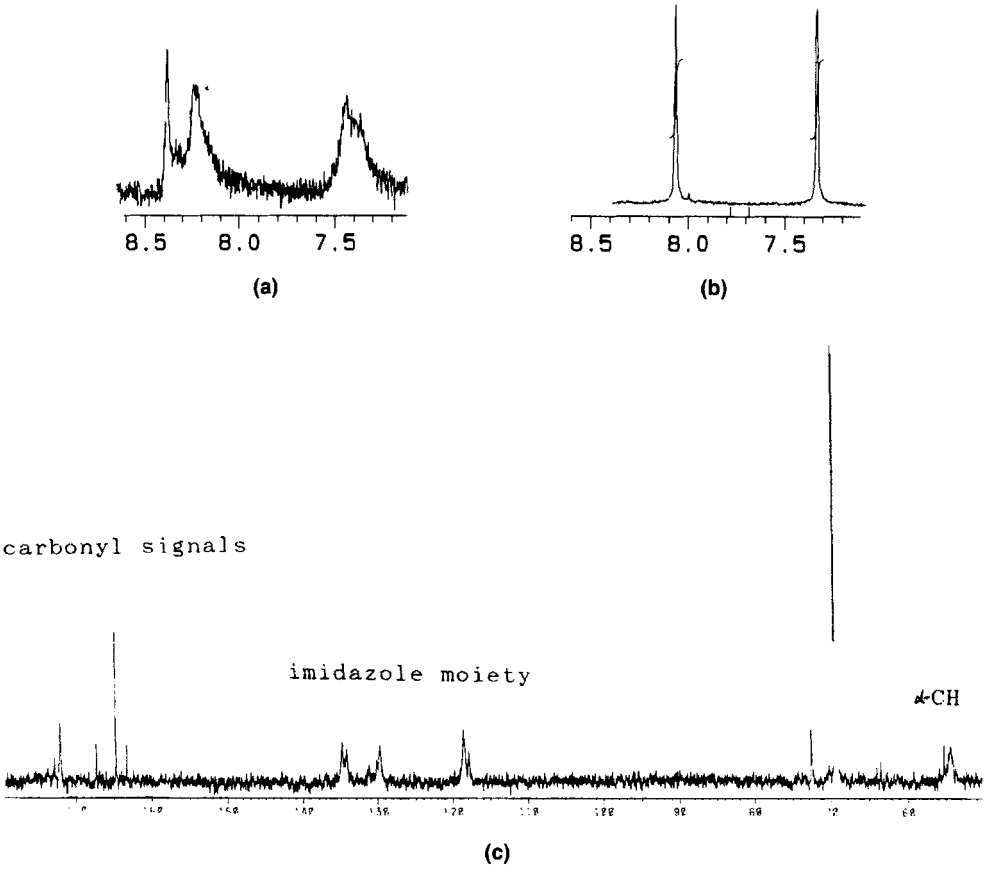
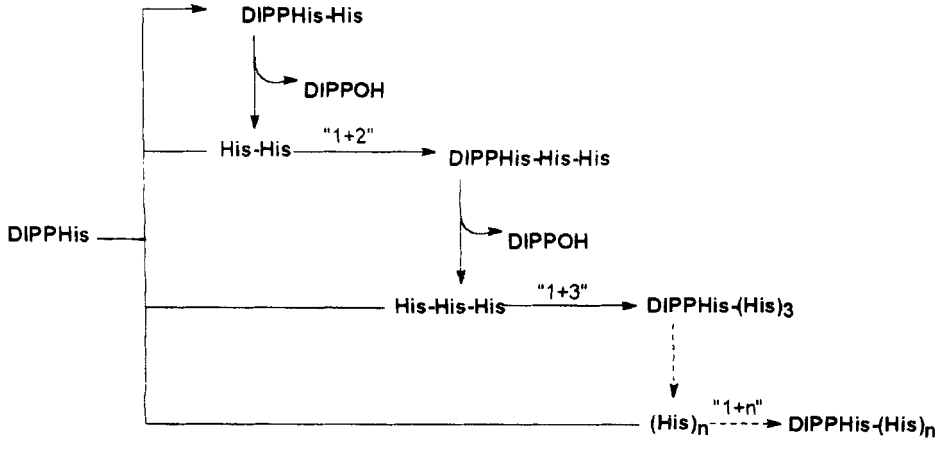
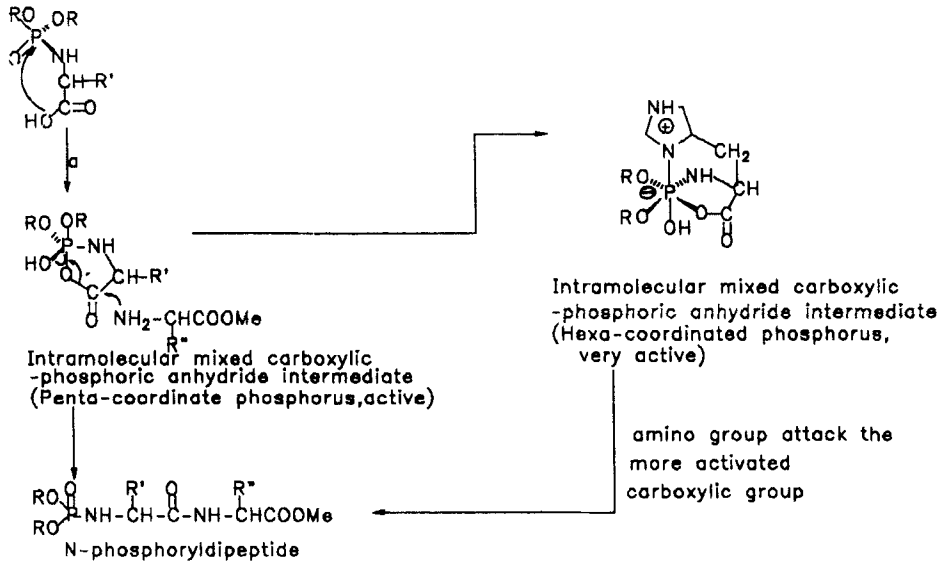


FIGURE 1 a) ^{1}H NMR spectrum for imidazole moiety of crude products derived from DIPPHis, b) ^{1}H NMR spectrum for imidazole moiety of histidine, and c) ^{13}C NMR spectrum of crude products derived from DIPPHis.



SCHEME III The "1 + n" mechanism for the oligopeptides formation.



SCHEME IV

results and some other finding,[5,7] it seems that an intramolecular mixed carboxylic phosphoric anhydride intermediate may exist in the reaction process (Scheme IV).

CONCLUSION

Our investigation found that the phosphoamino acids could not only activate themselves to give the peptides in the non-aqueous environment,[5,6] but also possess similar properties in the aqueous solution. Since the biological reaction usually happened in water solution, hence, these result might be responsible for the properties of phosphoamino acids in the living chemistry. The participation of the phosphoryl group may be the clue to the function of the phosphorylated protein in many important bioprocesses. It might be important for the prebiotic synthesis of a protein.

EXPERIMENTAL

Methods

^{1}H and ^{31}P NMR spectra were recorded on a Bruker AC 200 MHz and ^{13}C NMR on Bruker AM 500 MHz spectrometer. Positive and negative ion FAB-MS data were obtained on a KYKY Zhp-5 double-focusing mass spectrometer from Scientific Instrument Factory, Beijing, China, equipped with a standard KYKY fast-atom gun. IR spectra were measured as KBr disc on Nicolet 5DX FT-IR spectrometer.

Preparation of the Phosphoamino Acids

The preparation of N-(diisopropylphosphoryl) amino acids (DIPPHis **1**, DIPPAla **2**, DIPPCys **3**, DIPPGlu **4**, DIPPLys **5**) were synthesized by a previously reported general procedure.[6,7]

Preparation of Mixture Peptides

5–10 mg N-diisopropyl phosphorylated amino acids (**1–5**) and 5–10 mg of other amino acids or amino acid esters were put into a small tube, and then added the solvent 0.2 ml. The solutions were kept at 38°C for 6 days. The solvent was removed under reduced pressure at room temperature from the reaction mixtures. The residues were analyzed by FAB mass spectra and IR spectra.

DIPPAlaAla

N-diisopropyl phosphoalanine (1 g) was incubated in water for a week at 38°C. The product was isolated to give DIPPAlaAla (10 mg, 1%) by Si gel column chromatography,[5] FAB-MS (MH^+) m/z: 325. ^{31}P NMR δ ppm: 7.00. ^1H NMR δ ppm ($CDCl_3$): 1.05 (m, 15H), 2.52 (t, 2H), 3.22 (t, 2H), 3.87 (m, 1H), 4.60 (m, 3H), 7.15 (d, 1H), 8.90 (br, s, 1H). ^{13}C NMR δ ppm: 18.3, 37.5 (J = 3.4 Hz), 38.0, 48.2, 171, 3, 175.5. The spectra data were identical with an authentic sample.[8]

Identification of the Product of DIPPHis

N-diisopropyl phosphohistidine (1 g) was incubated in water for a week at 38°C. The product was removed from the main histidine by precipitating with MeOH, the residue was isolated to give a crude component (ca. 20 mg) as above. The spectra analysis indicated that the component was a mixture of peptides and histidine (Figure 1).

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