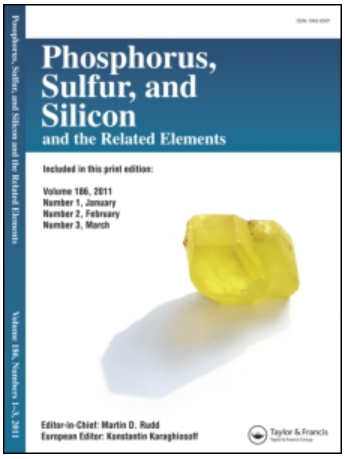

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FORMATION OF OLIGOPEPTIDES FROM N-PHOSPHOAMINO ACID BY INFRARED RADIATION

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Two types of oligopeptides: homopeptide DIPP-$(aa_1)_n$and heteropeptide DIPP-$(aa_1)_{n-1}$-aa_2 formed when the aqueous solution of N-phosphoamino acid (DIPP-aa_1) and amino acid (aa_2) was radiated by infrared light. An intramolecular mixed carboxylic-phosphoric-anhydride intermediate might exist in the peptide formation process.

Keywords: oligopeptide; N-phosphoamino acid; infrared radiated

INTRODUCTION

The phosphorus atom plays a crucial role in life chemistry, not only because phosphorus makes up 9% of nucleic acids, but also due to its regulative effect on enzyme activity (1). It is also worthwhile to note that more and more facts show that phosphoryl group might play an important role in the biosynthesis and prebiotic synthesis of peptide and protein (2–5). The self-activation reaction of N-phosphoamino acids to the peptide formation has been studied systematically in organic solvent (2–5). In this paper, the self-activation of N-phosphoamino acid to the oligopeptide formation in aqueous solution is reported. It is a self-catalysis process, which requires no catalysis or activator. The only condition is infrared radiation. It may be assumed that the phosphoryl group plays an activation role in the prebiotic synthesis of peptide and protein.

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RESULTS AND DISCUSSION

Introducing a phosphoryl group to the amino acid stimulated many interesting chemical properties. For example, it has been noticed that N-phosphorylated amino acids with side chain functional groups, such as the hydroxyl group, can self-activate to give the N→O migration product, esters and phosphoryl ester-exchanged products (6), so do the other amino acids with side chain functional groups.

Among those self-activating reactions, peptide formation reaction is the most interesting one. It was found that when N-diisopropylphosphoryl phenylalanine (DIPP-Phe) was incubated in organic solvent, such as $CHCl_3$, C_2H_5OH, etc., at room temperature for 15 days or more, the corresponding homodipeptide DIPP-Phe-Phe was detected (4). But if the phosphorylated amino acid was incubated at 40°C for 8–10 hrs, the corresponding homodipeptide was detected, eg. DIPP-Asp-Asp (2). When phosphoryl aspartic acid was incubated together with other amino acid ester or dipeptide ester, not only the homodipeptide, but also the corresponding heterodipeptide or tripeptide, such as DIPP-Asp-Cys·OBu, DIPP-Asp-Gly-Gly·OBu (5) were detected. Table I shows some typical types of the self-activating peptide formation reactions of N-phosphoamino acids. Some peptides were isolated and their structures were determined by ^{31}P-NMR, ^{1}H-NMR, ^{13}C-NMR, IR, FAB-MS and FAB-HRMS (2–5).

When N-phosphoamino acid was incubated in aqueous solution at 40°C, generally it was hydrolysized completely in 10–48 hrs (10). There is only little amount of peptides were detected (11). When N-phosphoamino acid was dissolved in aqueous solution and irradiated by infrared light, the temperature of the reactions was not controlled. But the final products were different. After the mixture was irradiated for about 8 hrs, the products are more complicated and more peptides were detected. For example, when DIPP-Ala and free Phe were mixed together in water and irradiated by infrared light for about 48 hrs, many spots could be found in TLC. From HPLC many peaks were detected with the retaining time 10.857 min, 8.925 min, 7.503 min, 5.380 min, 4.363 min, 3.565 min and 3.218 min respectively (Table II). The FAB-MS data show peaks at $(MH)^+/Z$=323, 399, 394, 470, 465, 541. These data and the other findings (2–5) strongly suggest the existence of DIPP-Ala- Ala, DIPP-Ala-Phe, DIPP-Ala-Ala-Ala, DIPP-Ala-Ala-Phe, DIPP-$(Ala)_4$, DIPP-$(Ala)_3$-Phe, respectively (Table III). The other mixtures have the similar results

(Table III). Some other peaks in FAB-MS also suggest the possibility of the existence of free oligopeptides (Table IV). These free oligopeptides might form in the self-activating reaction process or might be the successive phosphoryl group loss fragment ions of the corresponding phospho-oligopeptide.

TABLE I Typical types of the self-activating peptide formation reactions of N-phosphoamino acids

Type	*Example*					
	Entity	*Solvent*	*T (°C)*	*t (hr)*	*Products*	*Ref.*
"1+0"	DIPP-Asp	$CHCl_3$	40	15	DIPP-Asp-Asp	2
	DBP-Ala	C_4H_9OH	40–60	15	DBP-Ala-Ala	4
				48	DBP-$(Ala)_4$·OBu	4
					Ala-Ala	4
	DBP-Phe	AcOEt	25	15 days	DBP-Phe-Phe	4
"2+0"	DBP-Pro	Toluene	105	6	Pro-Pro	3
"1+1"	DIIP-His + Ala·OMe	DMSO-$CHCl_3$	40	16	DIPP-His- Ala·OMe	5
"1+2"	DIPP-His + GlyGly·OMe	DMSO-$CHCl_3$	40	16	DIPP-HisGlyGly·OMe	5
"2+1"	DBP-GlyGly + Tyr·OMe	Dioxane	120	16	DBP-GlyGlyTyr·OMe	7
	DBP-Ala-Tyr + Gly·OMe	Dioxane	120	16	DBP-AlaTyrGly·OMe	7

where: DIPP=Diisopropylphosphoryl - DBP=Dibutylphosphoryl.

TABLE II HPLC data of the residues

Entity	*Retaining Time (min)*						
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 5*	*Peak 6*	*Peak 7*
DIPP-Phe + Ser	2.392	2.967	4.453	8.660			
DIPP-Ala + Ser	3.175	4.962	5.848	8.110	9.117	10.906	
DIPP-Ala + Phe	3.218	3.565	4.363	5.380	7.503	8.925	10.857

Notes: Developping solvent: 50 mM KH_2PO_4: CH_3OH = 96: 4 (v/v). Flow rate: 1 mL/min. Detecting wavelength: 210 nm.

TABLE III FAB-MS data of the residues ($(MH)^+/Z$) **I**

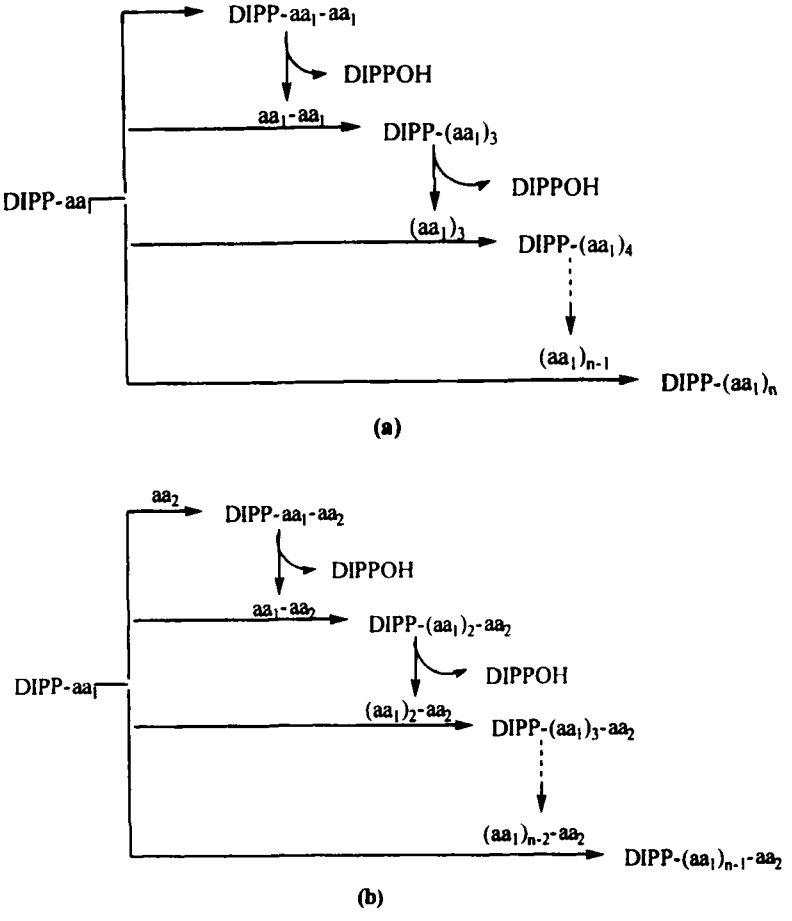
Entity		*Dipeptides*		*Tripeptides*		*Tetrapeptides*	
DIPP-aa$_1$	*aa$_2$*	*DIPP-(aa$_1$)$_2$*	*DIPP-aa$_1$-aa$_2$*	*DIPP-(aa$_1$)$_3$*	*DPP-(aa$_1$)$_2$-aa$_2$*	*DIPP-(aa$_1$)$_4$*	*DIPP-(aa$_1$)$_3$-aa$_2$*
DIPP-Ala	Phe	323	399	394	470	465	541
DIPP-Phe	Ala	475	399				
DIPP-Phe	Gly	475	385		532		
DIPP-Gly	Phe	295	385				
DIPP-Ser	Ala	355	339	442	426	529	
DIPP-Ala	Ser	323	339		410		481
DIPP-Ser	Thr	355	369	442			
DIPP-Thr	Ser	383	369				

TABLE IV FAB-MS data of the residues ($(MH)^+/Z$) **II**

Entity		*Dipeptides*		*Tripeptides*		*Tetrapeptides*	
DIPP-aa$_1$	*aa$_2$*	*(aa$_1$)$_2$*	*aa$_1$-aa$_2$*	*(aa$_1$)$_3$*	*(aa$_1$)$_2$-aa$_2$*	*(aa$_1$)$_4$*	*(aa$_1$)$_3$-aa$_2$*
DIPP-Ala	Phe	159		230		301	
DIPP-Phe	Ala	311	235				
DIPP-Phe	Gly	311	221	458	382		
DIPP-Gly	Phe	131	221				
DIPP-Ser	Ala	191	175	279	262	365	
DIPP-Ala	Ser	159					
DIPP-Ser	Thr	191	205	278			
DIPP-Thr	Ser	219	205	321	306		

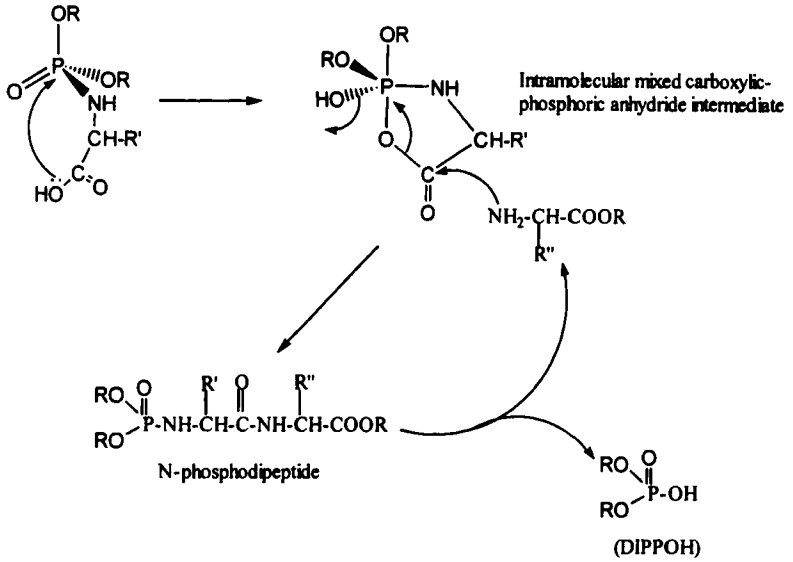
It is interesting to find that no matter what kind of entities used, the peptides formed are all in two types: DIPP-$(aa_1)_n$ or DIPP-$(aa_1)_{n-1}$-aa_2. Scheme 1 gives a simplified scheme to show the peptide formation process.

Lippmann (9) has proposed that the biosynthesis of protein proceeds through a mixed carboxylic-phosphoric-anhydride intermediate. These



SCHEME 1 (a) Process of homo-peptide formation (b) Process of hetero-peptide formation

experimental results, together with other findings (6), indicate that an intramolecular mixed carboxylic-phosphoric-anhydride intermediate might exist in the self-activating process of N-phosphoamino acid (Scheme 2). In this intermediate, the phosphoryl and carboxylic groups were promoted by each other. The activated carboxylic group was attacked by the amino group to form a new amide bond. In organic solvent, the intramolecular phosphoric-carboxylic anhydride was trapped and identified (5).



SCHEME 2 Mechanism of peptide formation from N-phosphoamino acids

How is the mechanism of the peptide formation reaction in the aqueous solution and radiated by infrared light? From the fact that only the two types of oligopeptides formed and the other findings (5), they might have the similar mechanism (Scheme 2).

CONCLUSION

It was found that when the aqueous solution of N-phosphoamino acid (DIPP-aa_1) and amino acid (aa_2) was irradiated by infrared light, the corresponding oligopeptides formed. There were only two types of oligopeptides: homo-peptide DIPP-$(aa_1)_n$ and hetero-peptide DIPP-$(aa_1)_{n-1}$-aa_2. An intramolecular mixed carboxylic-phosphoric-anhydride intermediate might exist in the peptide formation process. Therefore, it is phosphorus that promotes amino acids to form peptides, which might play an important role in the prebiotic synthesis of peptide and protein.

Experimental Procedures

Methods

^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra were recorded on a Bruker AM-200 spectrometer. Positive-ion FAB-MS data were obtained on a KYKY Zhp-5 double-focusing mass spectrometer from the Scientific Instrument Factory, Beijing, China, equipped with a standard KYKY fast-atom gun. FAB high-resolution mass spectral (FAB-HRMS) data were obtained on a double-focussing mass spectrometer (VG-ZAB-HS) and VG11–250 data system.

Preparation

The preparation of N-phosphoamino acids was carried out according to the literature (2–3,8). All physical constants and spectroscopic data of the products agreed with literature values.

Incubation of N-phosphoamino acids

To an N-phosphoamino acids (10 mmol), 20 mmol H_2O and 2 mmol NEt_3 were added. The mixture was radiated by infrared (infrared lamp) for 48 hrs. The residue was analyzed with TLC, HPLC and FAB-MS.

Chromatography

Analytical reversed-phase high performance liquid chromatogram was performed on Shimadzu LC-9A. 50 mM KH_2PO_4: CH_3OH = 96: 4 (v/v) was used to develop the chromatogram. The flow rate was 1 mL/min. The products were detected at 210 nm.

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