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Penta-coordinate phosphorous compounds and biochemistry

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Abstract The relationship between penta-coordinate phosphorus compounds and biochemistry is briefly reviewed. Some interesting phenomena such as peptide formation, ester formation, ester exchange on phosphorus and N to O migration occur at room temperature when the amino group of amino acid is associated with phosphoryl group. Serine or threonine in conjugate of nucleoside-amino acid could recognize different nucleobases. N-phosphoryl Histine and Ser-His dipeptide could cleavage nucleic acid, protein and ester in neutral medium. It is found that the above phenomena all undergo penta-coordinate intermediate of phosphorus atom, which is proposed as the key factor to determine their activities.

Keywords: penta-coordinate phosphorus compounds, biochemistry, nucleotide, amino acid, origin of life.

The origin of life on the earth is one of the most difficult scientific puzzles to be solved[1—3]. Where is the life from? How did the first organism come into being? It is still a question that needs us to explore. More and more experimental results show that the phosphorus plays an important role in the formation of biomolecules in prebiotic condition, such as amino acid, nucleoside, nucleotide, peptide, oligonucleotide and nucleic acid. The particular role of phosphorus in life science has attracted the attention of many scientists. Westheimer[4] expounded why nature chose phosphodiester from the view of point of organic chemistry. Todd[5], winner of the Nobel Prize, put forward the famous judgment “Where there is life, there is phosphorus”. Phosphorus was even chosen as a standard of the existence of life in space[6]. All of these greatly inspired us to explore how the biomolecule interacts with phosphorus, and evolves to the elaborate and complicated advanced organism.

In the study of chemical evolution, it might be important to synthesize some small organic molecules as simple biological model for studying the fundamental rule of life and exploring the origin of life. In the past two decades, organo-phosphorus chemistry has been quickly developed, many new reactions were discovered and novel phosphorus compounds were synthesized. The study of penta-coordinate phosphorus compound is of particular interest, as many bio-processes are found to perform via a penta-coordinate phosphorus intermediate, such as phosphoryl group migration of ATP, self-cleavage of RNA, phosphorylation and dephosphorylation of protein. It has been realized that these functions are associated with the particular molecular structure, orientation and rearrangement of ligand.

1 Biology significance of penta-coordinate phosphorus chemistry

1.1 Penta-coordinate phosphorus intermediate in the biological process of cAMP

cAMP and cGMP play very important roles in cell metabolism. According to the second messenger theory of Sutherland[7]: first, hormone binds to receptor of its target cell, and then cAMP in cell is generated, which could cause the concentration change of some chemical substances, or cause the structure change of some enzymes, and therefore the metabolism could be regulated. In this process, hormone is called the first messenger, and cAMP is called the second messenger. cAMP could interact with protein kinase as well as bind with phosphodiester, however, it was found that all these reactions undergo the penta-coordinate phosphorus intermediate which have the approximate geometry of trigonal bipyramid (TBP)[8,9].

1. 2 Penta-coordinate phosphorus intermediate in phosphoryl group migration

Phosphoryl group migration is one of the most important biological processes. It is also the key regulation step of various metabolism pathways inside cell. Many biological processes are carried out through the phosphoryl group migration, such as the oxygenolysis of glucose, activation of nucleoside in DNA and RNA synthesis process, biosynthesis of amino acid, and switch regulation of enzyme in signal transmission process[7]. Confirmed by the experiments[10,11], these phosphoryl group migrations also undergo the penta-coordinate phosphorus intermediate.

1.3 Penta-coordinate phosphorus intermediate in RNA hydrolysis process

As well known, DNA is the carrier of genetic information and RNA plays a very important role in the biosynthesis of protein. Compared with DNA, RNA is unstable and easy to be degraded. In room temperature, RNA could be quickly hydrolyzed in 0.1 mol/L sodium hydroxide solution; while DNA could be maintained for 1 h in 1 mol/L sodium hydroxide solution at 100℃ without degradation. The difference is the presence of 2′-hydroxy of RNA, whose adjacent participation results in the increase of hydrolysis of RNA. Breslow et al.[12] studied in detail the hydrolysis dynamics of UpU in imidazole buffer solution. It was found that the main active group of many RNA hydrolases such as cattle nuclease consists of two histidine residues, whose hydrolysis process undergoes penta-coordinate phosphorus mechanism.

1.4 Penta-coordinate phosphorus intermediate in hydrolysis of antiviral prodrugs

Most nucleoside analogues have antiviral activities, especially after conjugated with amino acid, not only its biological activity is enhanced but also its toxicity is decreased[13—15]. Studied on this phenomenon, the possible decomposition mechanism has been put forward[16]: methyl ester of amino acid is deesterized activated by esterase followed by oxygen atom attack on the penta-coordinate phosphorus atom, at the same time the amino acid is eliminated catalyzed by phosphodiesterase, finally the phosphate is generated.

2 Penta-coordinate phosphorus compound of amino acid and nucleoside

Nucleic acid and protein are two main substances of organism, and they perform the life cycle by inter-regulation and interaction. The key mechanism is the active participation of phosphoryl group. After systematic study, we proposed that “Phosphorus is the regulation center of life process”.

2.1 Chemical synthesis of penta-coordinate phosphorus compound

Phosphorus atom of penta-coordinate phosphorus compound is in dsp^3 hybridation. Compared with quat-coordinate phosphorus compound, penta-coordinate phosphorus compound has longer bond length and lower bond energy. The chemical bond is easier to break and to change into quat-coordinate state. Because of its chemical activity, it often appears as intermediate in the reaction process. So, it is very necessary to synthesize some penta-coordinate phosphorus compounds as the fundamental model for the study of their biochemistry. The penta-coordinate phosphorus compounds of amino acid, nucleoside and sugar have been synthesized in our lab[17].

2.2 Bio-mimic reaction of penta-coordinate phosphorus compound

2.2.1 Mechanism of peptide formation of N-phosphoryl amino acid. It was found that when amino acid is phosphorylated, some interesting phenomena occur at room temperature. It could perform peptide formation[18,19], ester formation[20], ester exchange on phosphorus[21,22], N to O and N to S migration[23–25], and oligonucleotide formation[26] (see fig. 1). Phosphorus atom being

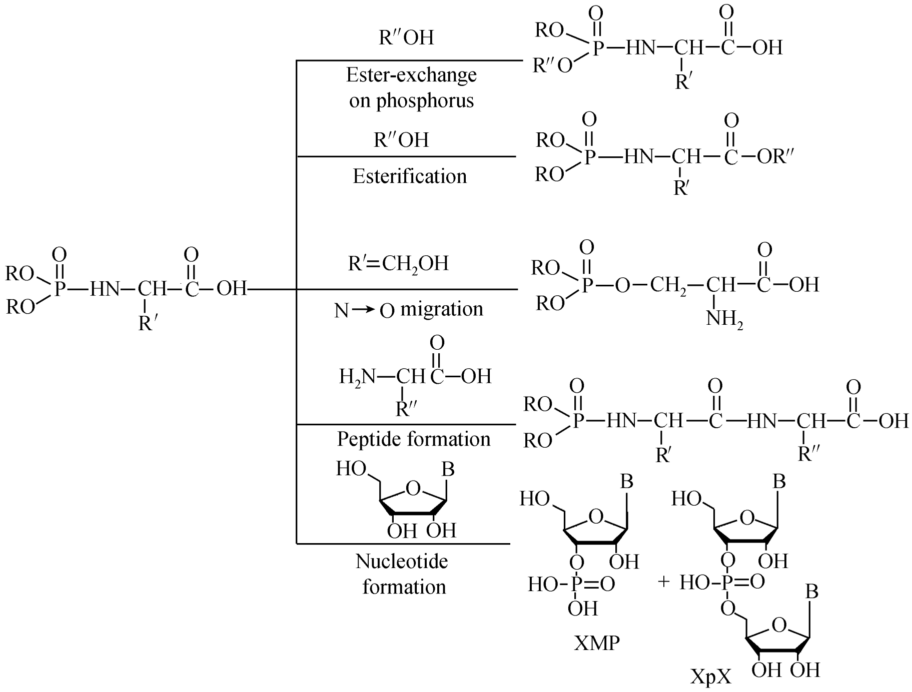
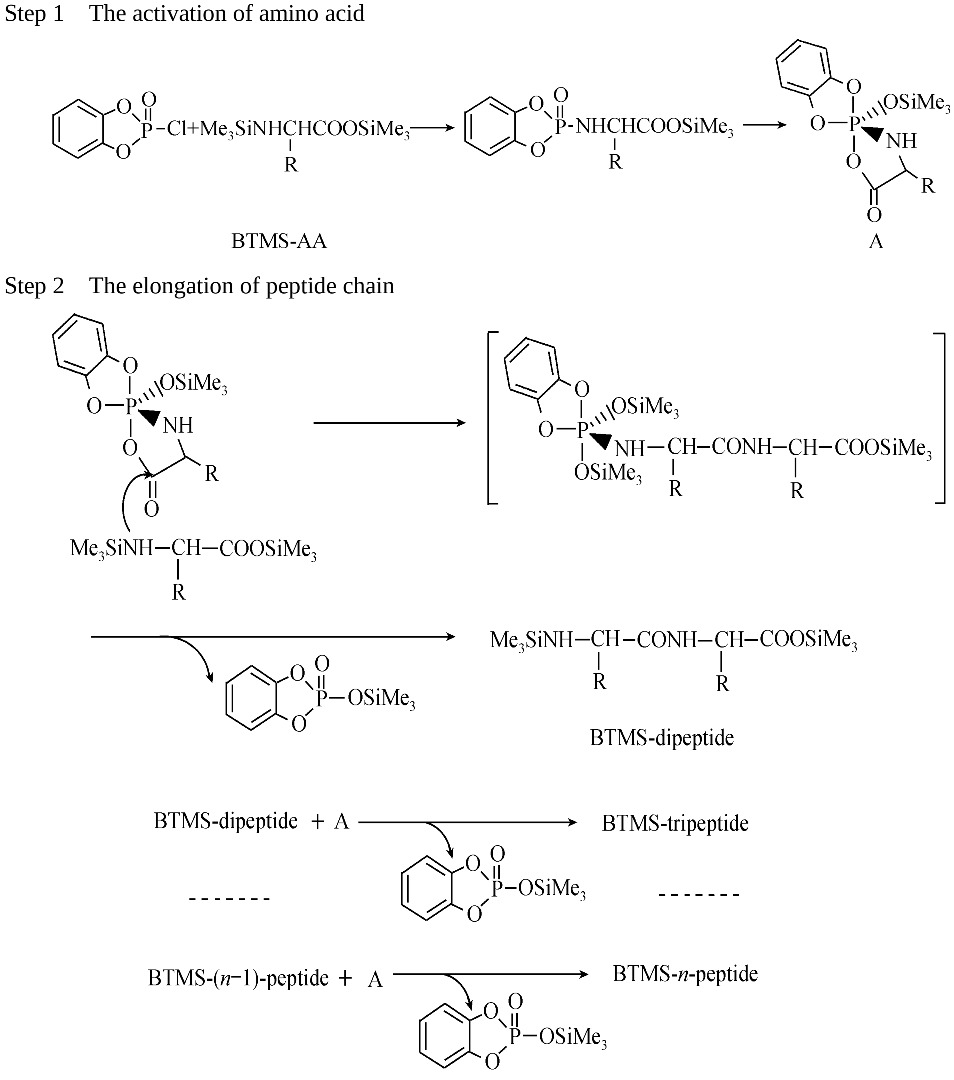


Fig. 1. Chemistry reaction of N-phosphoryl amino acid.

the center, and carboxyl group and side chain group participating the reaction at the same time, this is similar to the function of enzyme. Therefore, we call the N-phosphoryl amino acid and small peptide as “mini-type activate enzyme”.

It was presumed that it might undergo penta-coordinate phosphorus intermediate of amino acid in these reaction processes. However, the intermediate is unstable in water and alcohol, and only exists as instantaneous intermediate. In order to prove the reaction mechanism, we protected amino acid with chlorotrimethylsilane mediated by *o*-phenylene phosphorochloridate. The activated amino acid is a five-membered cyclic penta-coordinate 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 leads to the formation of peptide with release of a phosphate ester[27]. The mechanism is shown in fig. 2.



Step 3 The termination of peptide elongation

$$\text{BTMS-(di}\sim n\text{)-peptide} \xrightarrow[-\text{Me}_3\text{SiOH}]{\text{H}_3^{+}\text{O}} \text{(di}\sim n\text{)-peptide}$$

Fig. 2. Possible mechanism of self-assemble into peptide for N,O-BTMS-AA mediate by PPc.

2.2.2 Recognition of N-phosphoryl group on α-, β- and γ-amino acids. Under the condition of pribiotic earth, different amino acids could exist. Why the nature chose the α-amino acid is still a puzzle. After studying the character of phosphorylated α-, β- and γ-amino acids, it was found that only the α-amino acid could be activated to form peptide, while the β- and γ-amino acids are very stable under the same condition[27]. We proposed two penta-coordinate phosphorus intermediates (a) and (b) which constitutes with five-membered ring and six-membered ring respectively (see fig. 3).

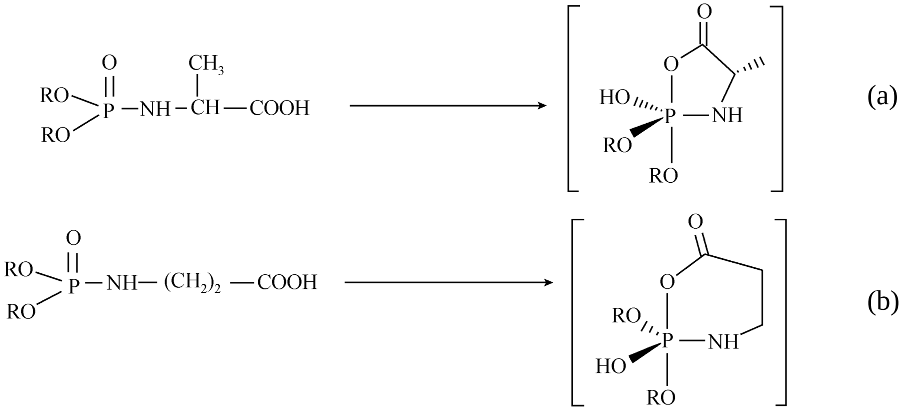


Fig. 3. Proposed intermediate of α- and β-amino acids.

In order to understand the different reactivities between N-phospho-α-amino acid and N-phospho-β-amino acid, the two penta-coordinate phosphorus intermediates have been investigated by density function theory. The structures and relative potential energy of the two intermediates were obtained by fully optimization under B3LYP/6-31G** level by Gaussian98 (see fig. 4). The relative potential energy of intermediate from DIPP-α-Ala is 45.61 kJ·mol^{-1} lower than that of the intermediate from DIPP-β-Ala. The result clearly shows that N-phospho-α- and β-amino acids have different reactivities.

2.2.3 Ester exchange reaction of penta-coordinate phosphorus compound. Ester exchange reaction could take place between penta-coordinate phosphorous and alcohols. If the alcohols generated from the reaction are removed from the system, the reactant could be completely converted to the product. If it reacts with 1,2-diols or 1,3-diols, it could produce penta-coordinate phosphorus compound containing five- or six-membered ring. Forming of the ring makes the groups around the phosphorus atom less "crowded", leading to the cyclic penta-coordinate phosphorus compound more stable than the acyclic one.

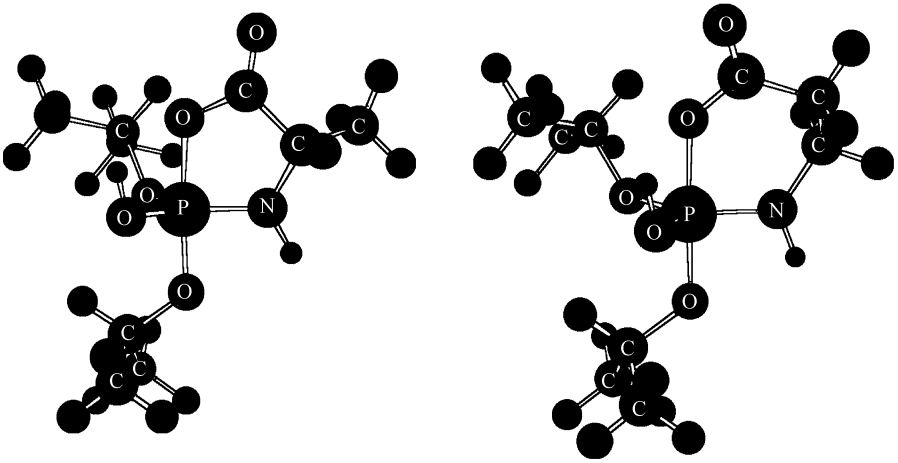


Fig. 4. Molecular model of penta-coordinate phosphoryl α- and β-amino acids.

The ester exchange reactions of single ring compound, 2,2,2-trimethoxy-4,5-dibenzyl-1,3,2-dioxaphosphorane, with series of simple alcohols in pyridine have been studied in our lab systematically[28](fig. 5). It is found that the rate of ester exchange with glycol is much faster than that of other alcohols. It is shown that forming of five-membered ring is very important to the stability of penta-coordinate phosphorus. The rate of ester exchange with glycol is 6 times faster than that of 1,3-diols. This special chemical selectivity of penta-coordinate phosphate containing five-membered ring is the structure factor that RNA is 10^6 times more active time than DNA, which is also the molecular foundation that RNA is the primer for DNA synthesis.

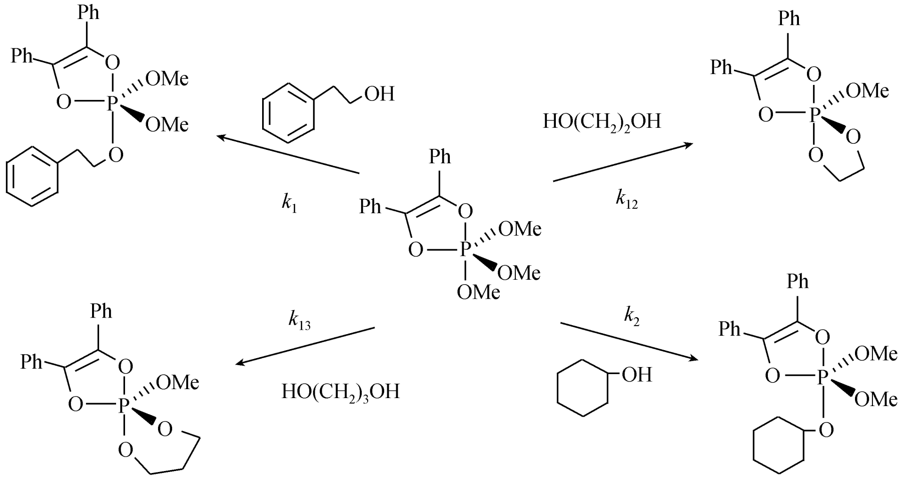
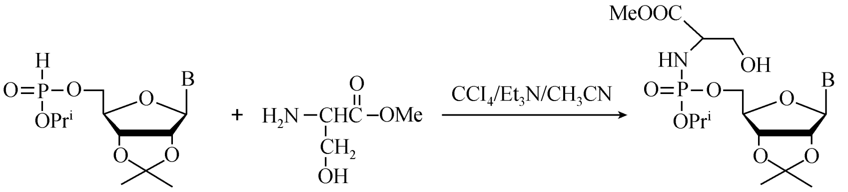


Fig. 5. Ester exchange reaction of penta-coordinate phosphorus compound with alcohols. $k_{12} : k_{13} : k_1 : k_2 = 100 : 25 : 16 : 1$, 25℃, 0.4 mol/L, Py.

2.2.4 Molecular recognition of nucleobase by amino acid. We synthesized the conjugate of amino acid methyl ester and nucleoside by modified Atherton-Todd reaction. The synthetic route is shown in fig. 6.



1a: B=A, **1b**: B=U
1c: B=C, **1d**: B=G

2a: Ser-AMP, **2b**: Ser-UMP
2c: Ser-CMP, **2d**: Ser-GMP

Fig. 6. Synthesis of serine 5′-phosphoamidate of nucleoside.

It was found that CMP-, GMP- and UMP-serine conjugate shows distinct chemical property compared with the corresponding AMP derivative. When treated with one equivalent of NEt_3, Ser-5′-AMP (**2a**), O-isopropyl O-(2′, 3′-isopropylidene) adenosin-5′-yl N-phosphoryl serine methyl ester, is stable at 25℃ for one week in anhydrous CH_3CN. However, the corresponding Ser-5′-UMP (**2b**), Ser-5′-CMP (**2c**) and Ser-5′-GMP (**2d**) are completely dephosphorylated at 25°C after 48 h in the same reaction media (see fig. 7).

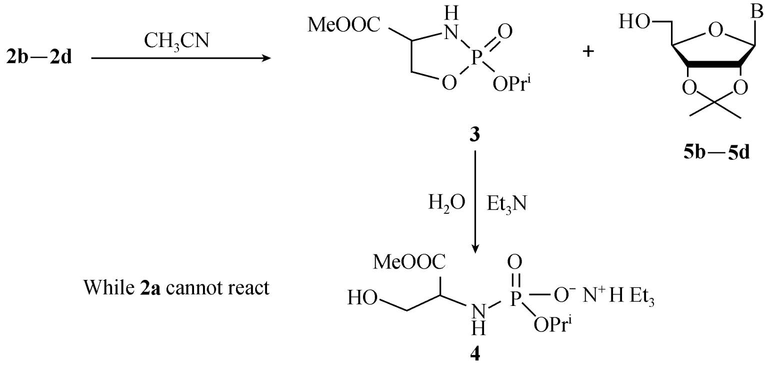


Fig. 7. Dephosphorylation of serine conjugated 5′-UMP, CMP and GMP (**2b**—**2d**).

The mechanism for intramolecular dephosphorylation of serine-conjugated-5′-UMP (**2b**) is proposed in fig. 8. There are a pair of hydrogen bonds between the hydroxyl group of serine and C2-carbonyl group of uracil base, which causes a switch of pyrimidine from anti- to syn-conformation. By this conformation, hydroxyl group of serine attacks the phosphorus to elevate it into a penta-coordinated intermediate which in turn would be dephosphorylated to release nucleoside (**5b**). For serine-conjugated 5′-CMP and GMP (**2c, 2d**), similar mechanisms are also proposed for their dephosphorylation reactions. This result provides a very good example for the interaction between nucleobase and amino acid side chain.

2.3 Ser-His dipeptide as a nucleic acid cleavage agent

In the past twenty years, artificial nuclease has received considerable attention for its very important applications in chemistry, biochemistry and molecular biology. By studying the artificial nuclease, we can obtain some significant clues to understand the catalytic mechanism of natural nuclease and push forward the development of genetic engineering. So it is important to design and synthesize some organic molecules and use them as nuclease model to selectively recognize and cleavage nucleic acid.

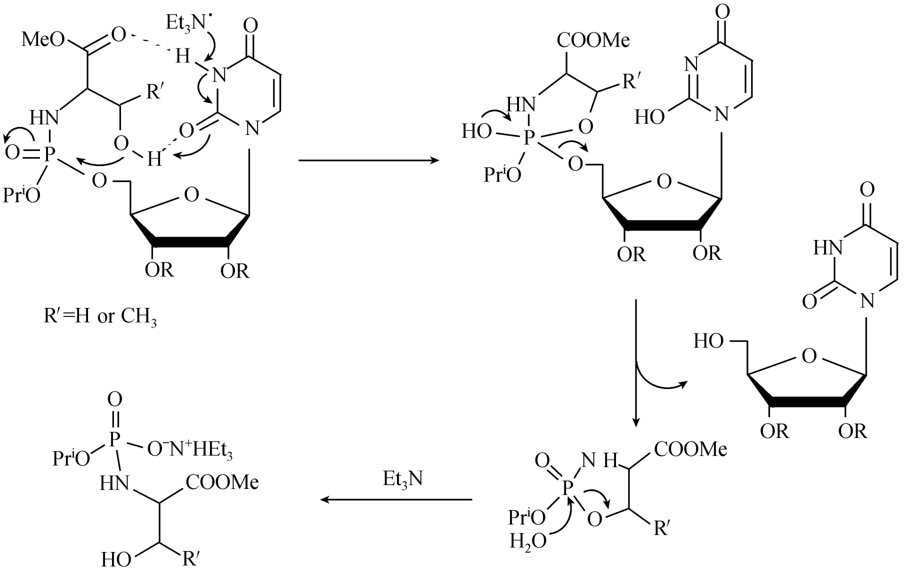


Fig. 8. Proposed catalytic mechanism of the dephosphorylation of serine-conjugated 5′-UMP, CMP and GMP (**2b**—**2d**).

It was found that phosphoryl histidine and Ser-His dipeptide could cleavage nucleic acid, protein and ester in neutral condition[29,30]. Especially for Ser-His dipeptide, it is the smallest peptide that could cleavage various natural substrates so far, and it is also the first reported DNA hydrolysis agent containing no mental ion.

The mechanism of Ser-His dipeptide cleavaging DNA is proposed as follows: protonized amino group, imidazole group and amide bind with phosphodiester backbond by hydrogen bonds, then the hydroxyl group of Ser attacks phosphorus atom to hydrolyze the phosphodiester via a penta-coordinate phosphorus intermediate, then DNA is cleavaged. The pathway (i) (see fig. 9) produces oligonucleotide containing 3′-hydroxy, and pathway (ii) produces oligonucleotide phosphodiester containing 3′-hydroxyl. The possible mechanism that Ser-His dipeptide cleavages DNA is shown in fig. 9.

2.4 Model of “protein and nucleic acid co-evolution”

After long and systematic study on phosphorus chemistry, especially the function of penta-coordinate phosphorus in life science, Zhao[31—33] proposed a theory of “protein-nucleic acid co-evolution” as shown in fig. 10. She suggested that peptide and nucleic acid are co-originated from phosphoryl amino acid—— the reaction product of high energetic phosphorus compounds with amino acid. As the volcano activities on primitive earth were frequent, pyrophosphate and pyrophosphate salts were probably accumulated on the earth’s surface. Through the intermediate process to form P-N bond with amino acid, the high energy contained in the P-O-P bond of these compounds could be transferred to peptide bond and phosphodiester bond of nucleoside. Therefore, N-phosphoryl amino acid could be considered as a reaction model to study many processes of life origin, such as chiral origin and genetic code origin. This viewpoint has attracted popular attention[34].

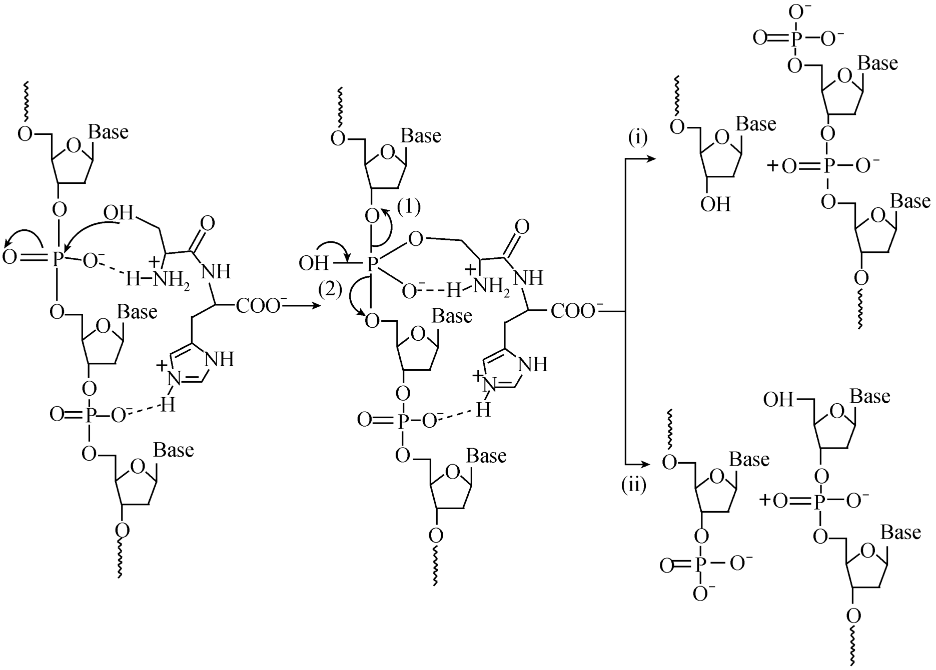


Fig. 9. Possible DNA cleavage mechanism catalyzed by Ser-His dipeptide.

3 Conclusions

Hence, it is concluded that phosphorus plays an inevitable role in both the life evolution and life process. Further studying on phosphorus chemistry will be helpful to elucidate their corresponding chemical mechanisms and get insight into the basic principle of life. Recently, the work on penta-coordinated phosphorus represents a totally new trend for the bioorganic phosphorus chemistry. More and more literatures have shown that an inter-discipline of dealing with high coordinated phosphorus is formed, which involves in the organic synthetic chemistry, physical organic chemistry, computational chemistry, crystallography, modern spectrum and molecular biology, etc. The above researches are focused on the exploration to the phosphorus-related enzymatic mechanisms. Especially, the increase in coordination of phosphorus from the nearby residues has important implication for enzymatic mechanistic studies of nucleophilic displacement reactions of the phosphoryl transfer enzymes[35]. This mechanism depicts phosphorus in an activated state intermediate with a trigonal bipyramidal (TBP) geometry. With the evidence to indicate the above-mentioned penta-coordination intermediate mechanisms, gradually, the so-called mechanism-based inhibitor design for the corresponding phosphorus-related enzyme such as RNase[36] or

Kinase[37] is coming to stage and some positive results are academically reported.

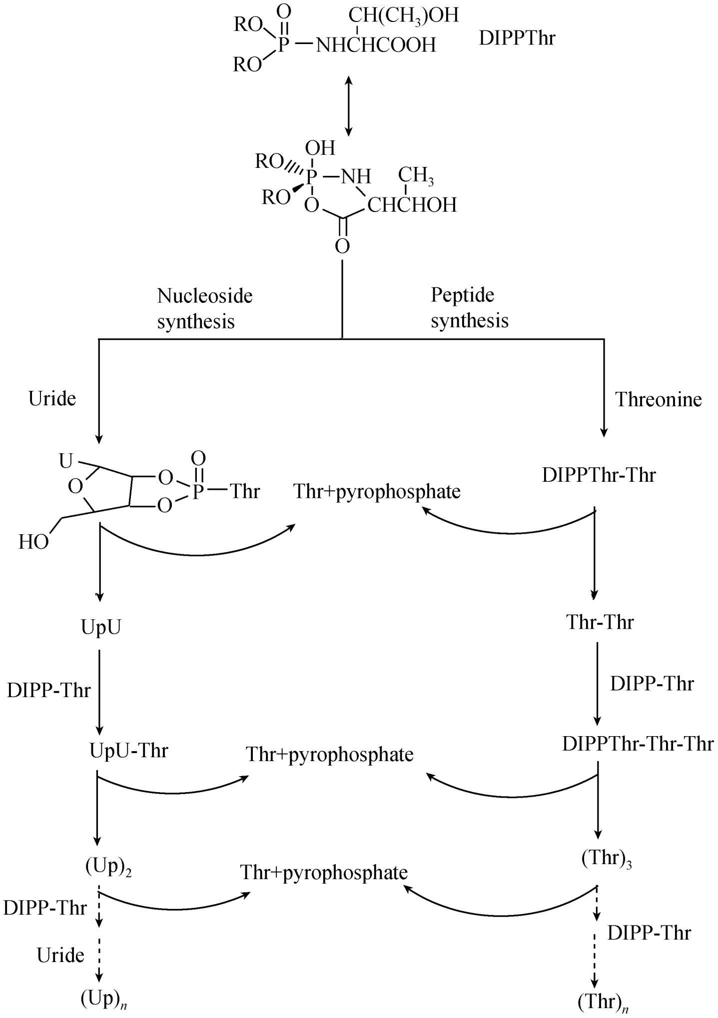


Fig. 10. Model of protein and nucleic acid co-evolution.

In our research group, most work will continue to be focused on the high-coordinated phosphorous chemistry, particularly, on its biological significance. And hopefully, the interaction mechanism between nucleic acid and phosphoryl protein will be elucidated in sense of penta-coordinate phosphorus intermediate.

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