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Theoretical Study on the Activity of a -COOH and b-COOH of *N*-Phosphoryl Aspartic Acids

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Abstract: The bio-mimic reactions of *N*-phosphoryl amino acids are very important in the study of many biochemical processes. The difference of reactivity between α-COOH and β-COOH in phosphoryl aspartic acid was studied by theoretical study (Hartree-Fock and Density Functional methods) in this paper. The intermediates **II** containing five-membered ring were more stable than **III** with six-membered ring. While for intermediates **III**, the isomers with six-membered ring in apical-equatorial spanning arrangement were more stable than those with di-equatorial spanning arrangement. At B3LYP/6-31G** level, it was shown that transition states **IV** and **V** involving α-COOH or β-COOH group had energy barriers of *DE* = 58.67 $kJ \cdot mol^{-1}$ and 103.94 $kJ \cdot mol^{-1}$, respectively. These results were in agreement with the experimental data. So the α-COOH group was involved in form of the intramolecular penta-coordinate phosphoric-carboxylic mixed anhydride intermediates, but not β-COOH group.

Keywords: α-Carboxylic group, β- carboxylic group, theoretical study, *N*-phosphorylation.

Many biological processes, such as signal transduction and protein synthesis are regulated by the phosphorylation and dephosphorylation of amino acid residues in the proteins[1]. In addition, formation of high-coordinate phosphoric intermediates is usually described as a key step in most of enzyme catalytic mechanisms[2]. Our previous work indicates that *N*-phosphoryl amino acids and peptides are chemically active species that characterize the bio-mimic reactivity[3], which might be related to the phosphorylation and dephosphorylation of proteins[4] and the intramolecular penta-coordinate carboxylic-phos-phoric mixed anhydrides were proposed as the common intermediates for the related reactions[5,6].

Since aspartic acid has both α-COOH and β-COOH, it is important to clear up which carboxylic group is involved in the formation of penta-coordinate phosphoric intermediate. In our previous paper, the activity difference between α-COOH and β-COOH was studied by MNDO method[7]. In this paper, Hartree-Fock(HF) and Density Functional (DFT) methods were adopted to study the mechanism of forming penta-coordinate phosphoric intermediates from *N*-phosphoryl aspartic acid and compare the activities of α- and β-carboxylic group. According to our previous results[3-6], *N*-dimethylphosphoryl (DMP) L-aspartic acid **I** was selected as the model reactant.

At B3LYP/6-31G** level, the geometry of DMP-Asp **I** was close to the crystal

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structure of *N*-diisopropylphosphoryl (DIPP)[8] alanine which had been reported by our laboratory. Comparing the two anions of DMP-Asp, it was shown that α-CO_2^- anion was 26.54 kJ/mol more stable than β-CO_2^- anion. From the charge distribution of α-CO_2H and β-CO_2H in DMP-Asp, proton might be easier to dissociate from α-CO_2H than from β-CO_2H. As a result, the formation of the penta-coordinate phosphoric intermediates from α-COOH group might become easier.

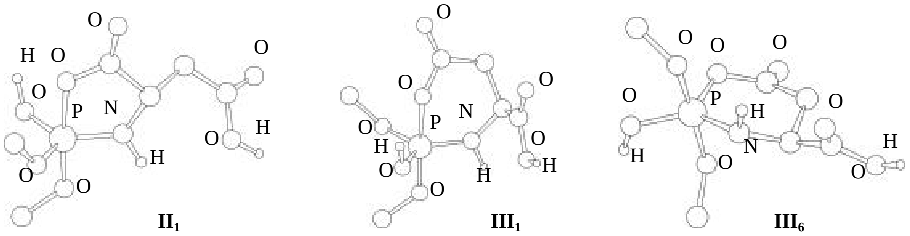
Comparing Penta-Coordinate Phosphoric Intermediates II and III

Table 1 Relative energy (R E) of the intermediates at HF/6-31G** level

Compounds	R E (kJ/mol)	Compounds	R E (kJ/mol)	Compounds	R E (kJ/mol)
II$_1$	44.60	**III$_1$**	87.09	**III$_4$**	170.87
II$_2$	67.00	**III$_2$**	99.87	**III$_5$**	171.52
II$_3$	60.15	**III$_3$**	104.51	**III$_6$**	161.88

[*] **II** and **III** stand for intermediates containing five or six-member ring respectively. Hydroxyl group is at the same side of the rest carboxylic group for **1** and **4**, at the different side for **2** and **5**, on the ring plane for **3** and **6**.

Figure 1 The optimized structures of II$_1$, III$_1$ and III$_6$ at B3LYP/6-31G** level



II$_1$ **III$_1$** **III$_6$**

The penta-coordinate phosphoric compounds II and III prefer trigonal bipyramidal configurations. There may exist two types of penta-coordinate phosphoric intermediates, containing a five-membered ring formed by α-carboxylic group or a six-membered ring formed by β-carboxylic group, respectively. For penta-coordinate phosphoric intermediates II, five-membered ring prefers apical-equatorial posture because of angel strain and ring strain. While for III, six-membered ring might be apical-equatorial posture or di-equatorial posture[9]. For apical-equatorial posture, from experiments and theoretical computations[10], the isomers with phosphoric-carboxylic mixed anhydride bond in apical position are more stable than those in equatorial position. So the latter were not considered in this paper.

For intermediates II, there were three isomers (**II$_1$**-**II$_3$**), while six isomers (**III$_1$**-**III$_6$**) for intermediates III. The nine positional isomers were optimized at HF/6-31G** level and the relative energy were shown in **Table 1**. It was shown that isomers **II$_1$**, **III$_1$**, and III$_6$ were most stable among the corresponding intermediates. For intermediates III, the isomers with six-membered ring in apical-equatorial spanning arrangement were 60 kJ/mol lower than those with di-equatorial spanning arrangement. The energy difference between the two arrangements was large so the intermediates III should be considered in the apical-equatorial spanning arrangement. The isomers II with five-membered ring had lower energy than III. For each kind of optimized isomers, it was found that the most stable structures were those in which the hydroxyl group and the rest carboxylic group of aspartic acid were at the same side of the ring, which can reduce the steric hindrance of phosphorus atom.

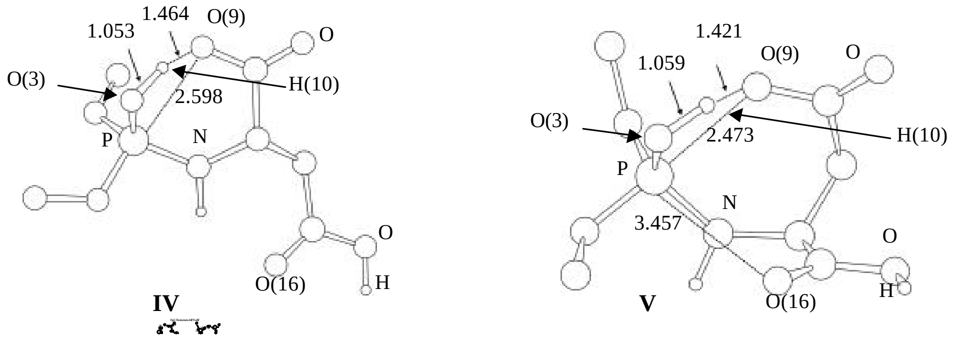
At B3LYP/6-31G** level, the optimized geometry of II_1, III_1 and III_6 were depicted in **Figure 1**. From the geometry, it can be seen that intermediates II and III were twisted trigonal bipyramid, which were in agreement with that predicted by the reference[11]. For II_1, the atoms in five-membered ring were almost coplanar. The six-membered ring in III_1, was in a boat form, while in a chair form for di-equatorial spanning arrangement III_6.

At the HF/6-31G**, B3LYP/6-31G** and B3LYP/6-311+G**// B3LYP/6-31G** level, the energy difference ΔE **(**III_1–II_1**)** with methyl group on phosphorus atom was in accordance with those with isopropyl group very well. In addition, the geometry of phosphoryl aspartic acid, II_1 and III_1 with isopropyl group were almost the same as those with the methyl group. So the simplification was reasonable and can save the CPU time.

Geometry of the Transition States and the Possible Mechanisms

Isomers II_1 and III_1 were appropriate isomers for searching for the corresponding transition states by STQN method. The transition states IV and V were fully optimized at B3LYP/6-31G** level. At B3LYP/6-31G** level, their geometry were shown in **Figure 2**. The frequency calculations showed that IV or **V** had only one imaginary frequency and the imaginary frequency corresponding to proton H(10) transferring and bond P-O(9) formation. So the transferring of proton H(10) to oxygen O(3) and the formation of bond P-O(9) were cooperative in the forming of penta-coordinate phosphoric intermediates.

Figure 2 The optimized structures of IV and V at B3LYP/6-31G** level



In the pathway of forming II_1, the positive activation energy is 58.67 kJ· mol^{-1} and the inverse activation energy is 42.86 kJ mol^{-1} in gas phase. The whole reaction of forming penta-coordinate phosphoric intermediate II_1 is endothermic. While in the pathway of forming III_1, the positive activation energy is 103.94 kJ· mol^{-1} and the inverse activation energy is 47.02 kJ mol^{-1} in gas phase. So the energy barrier of forming IV was 45.27 kJ mol^{-1} lower than forming V. So the α-COOH products are more favored thermodynamically and kinetically. This result was consistent with the experimental fact that only α-unprotected amino acids could take bio-mimic reactions[6].

Conclusion

The calculations were carried out at the HF/6-31G**, B3LYP/6-31G** and single-point B3LYP/6-311+G**//B3LYP/6-31G** level. The model that isopropyl group on phosphorus atom was simplified by methyl group was reasonable. From the calculations, the intermediates II containing five-membered ring were more stable than III with six-membered ring. Intermediates III with apical-equatorial spanning arrangement were more stable than those with di-equatorial spanning arrangement. The theoretical calculations were in accordance with the experiments very well. So α-COOH activates phosphoryl group intramolecularly by forming a five-membered ring phosphoric-carboxylic mixed anhydride intermediate, while the six-membered analogue from β-COOH group is unfavorably in the account of energy.

Acknowledgments

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