Theoretical Prediction of the ^{31}P NMR Chemical Shift of the Hexa-Coordinate Phosphorus Intermediate for the Phosphoryl Ester Exchange and N → O Migration Reactions of Dimethyloxyphosphoryl-Threonine

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Ab initio GIAO magnetic shielding calculations have been carried out on the hexa-coordinate phosphorus intermediate and the corresponding reactant and product for the N → O migration reaction of dimethyloxyphosphoryl-threonine. The calculated results lead us to predict that the ^{31}P NMR chemical shift of the hexa-coordinate phosphorus intermediate is -138 ppm, while the ^{31}P chemical shifts of the corresponding reactant and product are 4.9 ppm and -6.6 ppm, respectively.

1a: R'= CH$_3$; R"= CH$_3$
2a: R'= isopropyl; R= isopropyl

1b: R'= CH$_3$; R"= CH$_3$

1c: R'= CH$_3$; R"= CH$_3$
3c: R'= isopropyl; R"= n-butyl

Phosphorylated protein plays a vital role in many biological aspects because a large number of biological processes, such as metabolism, muscle contractibility, membrane transport, the initiation on the genetic transcription, cell cycle control and protein synthesis, are regulated by the phosphorylation and dephosphorylation on serine, threoine or tyrosine residues in enzymes.[1-6] The cancer is also involved in the protein phosphorylation mechanism.[3] However, the intrinsic relationship between the phosphoryl group and the amino acid residues in the proteins is still not clear. Numerous experimental studies on the structure-reactivity relationship about the phosphorylated amino acids have been carried out in order to approach this problem, and much attention has been focused on the regulation effect of phosphoryl group on side chain of amino acid,[4-6] such as $(R'O)_2P(O) - NHCH(COOH)CH(R)OH$. The experimental results reveal an important fact that the side chains of amino acids act as the relay device to modulate the chemical reactivity of the N-phosphoryl amino acids. It is very interesting that all the experimental observations could be understood if and only if a special kind of hexa-coordinate phosphorus intermediate exists indeed in the reaction process of the dialkyloxyphosphoryl amino acid, as reported by Zhao *et al.*[4-6] In the proposed intermediate, two five-membered rings and a six-membered ring involve the phosphorus atom and a carbon atom, simultaneously, as two nodes of the three rings.[7] It looks, however, structurally very unstable, and its existence has not been confirmed by any direct experimental evidence.

To examine the possibility for the existence of this kind of hexa-coordinate phosphorus intermediate, we have carried out *ab initio* calculations[7] on the possible hexa-coordinate phosphorus intermediate (**1b**) in the migration reaction from reactant $(CH_3O)_2P(O) - NHCH(COOH)CH(CH_3)OH$ (**1a**) to product $(CH_3O)_2P(O) - OCH(CH_3)CH(NH_2)COOH$ ((**1c**). (See structures below) The geometry optimization and harmonic frequency calculation were performed at HF/6-31G** level, and the calculated results[7] reveal that the hexa-coordinate phosphorus intermediate is a stable structure because it is associated with a local minimum on the potential energy surface, which supports the hypothesis derived from the experimental work reported by Zhao *et al.*[4] By use of the calculated results, it is very easy to understand the reactivities
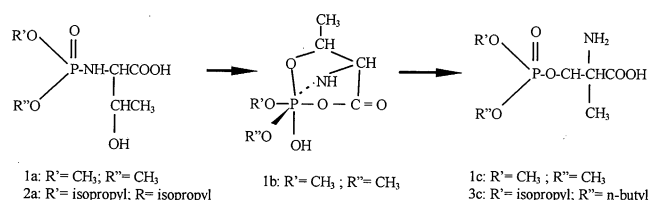
of dimethyloxyphosphoryl-threonine and other similar dialkyloxyphosphoryl amino acids.

Because of the difficulty for isolating this special kind of intermediate, it is very important to propose a method to directly observe and monitor the hexa-coordinated phosphorus intermediate in the reaction system. It is well-known that the ^{31}P NMR experiment is one of the most convenient techniques to characterize the organic phosphorus. However, the question is where the ^{31}P NMR signal of this kind of hexa-coordinate phosphorus intermediate will appear. How do we differentiate the ^{31}P NMR signals of the hexa-coordinate phosphorus intermediate from those of the reactant and product? Of course, from the reactant to the hexa-coordinate phosphorus intermediate, the ^{31}P NMR signal should have an upfield shift undoubtedly. The ^{31}P NMR chemical shifts of other kinds of hexa-coordinated phosphorus compounds collected in a recently published review[8] are between -77 ppm and -145 ppm. Nevertheless, the ^{31}P NMR chemical shifts of some penta-coordinated phosphorus compounds are also close to this region. For example, the ^{31}P NMR chemical shift of $P(OCH_2CF_3)_5$ was reported as -76.6 ppm.[9]

To predict the ^{31}P NMR chemical shift of the hexa-coordinate phosphorus intermediate described above, we have carried out *ab initio* magnetic shielding calculations with the gauge independent atomic orbital (GIAO) method using Hartree-Fock (HF) wave functions on **1b**, **1a**, **1c** and H$_3$PO$_4$ (as the reference of the ^{31}P NMR chemical shift). All the calculations were carried out at various levels by use of GAUSSIAN 94 package on NEC-SX3 supercomputer at the computer center of Institute for Molecular Science. The calculated chemical shifts relative to phosphoric acid are summarized in Table 1.

As seen in Table 1, the values of the ^{31}P NMR chemical shift of the intermediate calculated with GIAO method at HF/6-311G**//HF/6-31G** and HF/6-311+G**//HF/6-31G** levels are very close to each other. The difference between the two values of the chemical shift of the intermediate (**1b**) calculated at HF/6-311+G**//MP2/6-31G** level and at HF/6-311+G**//HF/6-31G** level is also only 0.2 ppm which can be ignored. These results indicate that for the GIAO calculations on the systems studied here, both the 6-311G** and 6-311+G** basis sets are large enough, and the HF/6-31G** geometry is not changed much by the electron

Table 1. ^{31}P NMR chemical shifts in ppm (relative to phosphoric acid) calculated by using GIAO method with various basis sets at geometries optimized at HF/6-31G** level except for the value in parenthesis which was calculated at the geometry optimized at MP2/6-31G** level

Approximation level	1a	1b(intermediate)	1c
HF/6-31G**		-109.7	
HF/6-311G**		-138.0	
HF/6-311+G**	4.9	-137.8(-138.0)	-6.6

correlation effect. Therefore, we finally carried out the GIAO calculations on the other systems at HF/6-311+G**//HF/6-31G** level.

From the results listed in Table 1 one can see that the ^{31}P NMR chemical shifts of **1a** and **1c** are calculated as 4.9 ppm and -6.6 ppm, respectively. According to these calculated results, the ^{31}P NMR signal of the **1c** should appear at high field for about 11.5 ppm relative to the **1a**. Our calculated ^{31}P NMR chemical shift of **1b** is about -138 ppm which is between -77 ppm and -145 ppm observed for other kinds of hexa-coordinated phosphorus compounds. From the **1a** to **1b**, the predicted ^{31}P NMR signal has a larger upfield shift of about 143 ppm.

It may be helpful for the estimation of the ^{31}P NMR chemical shifts of the hexa-coordinate phosphorus intermediates in the similar reactions to compare our calculated ^{31}P NMR chemical shifts of the **1a** and **1c** with the experimental data reported for the analogous dialkyloxyphosphoryl amino acids.[4] The experimental ^{31}P shift of N-diisopropyloxyphosphoryl-threonine (**2a**) was reported as 6.1 ppm, which is very close to our calculated ^{31}P NMR chemical shift 4.9 ppm of **1a**. The experimental ^{31}P shift of O-butyloxyisopropyloxyphosphoryl-threonine (**3c**) was reported as -2.8 ppm which can reasonably compare with our calculated ^{31}P NMR chemical shift -6.6 ppm of **1c**. The experimental upfield shift of the ^{31}P NMR signal from **2a** to **3c** (see structures of **2a** and **3c** shown above) is 8.9 ppm which is reasonably close to our calculated upfield shift from **1a** to **1c** although the amount of the

upfield shift becomes slightly smaller when the alkyl in the dialkyloxyphosphoryl-threonine becomes larger. With this in mind, we may expect that the ^{31}P NMR chemical shifts of the same kind of the hexa-coordinate phosphorus intermediates in the reactions of the analogous dialkyloxyphosphoryl-threonine may be also close to -138 ppm. It is expected that this prediction will help the experimentalists to look for the signals and to confirm the reaction mechanism by a further experimental NMR study using a powerful high-resolution ^{31}P NMR technique.

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