

^{31}P NMR and ESI-MS Study of Fenitrothion-Copper Ion Complex: Experimental and Theoretical Study

Hojune Choi, Kiyull Yang,* Jong Keun Park, and In Sun Koo*

Department of Chemistry Education and Research Institute of Natural Science, Gyeongsang National University, Jinju 660-701, Korea. *E-mail: iskoo@gnu.ac.kr
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^{31}P NMR and ESI-MS studies of Cu^{2+} binding to Fenitrothion (FN) were performed by experimentally and theoretically. The calculated ^{31}P NMR chemical shifts for FN- Cu^{2+} complexes are in good agreement with experimental chemical shifts in order, and the results present an important information for organophosphorus pesticide metal complexes. ESI-MS and low energy CID MS/MS experiments of FN- Cu^{2+} complexes combined with accurate mass measurements give insight into the metal localization and allow unambiguous identification of fragments and hydrolysis products.

Key Words: Fenitrothion (FN), Electrospray ionization mass spectrometry (ESI-MS), Density functional theory (DFT), Cu^{2+} -FN complex

Introduction

The organophosphorus (OP) pesticides have been known as fatal chemicals and have proven strong dependence on the chemistry of their aquatic environmental.¹ Due to their biological and environmental importance, the degradation of OP compounds has been studied extensively over the past several decades.² Fenitrothion (FN) is remarkably less toxic to mammals than other organophosphate insecticides such as methyl parathion and parathion. For this reason, it is used extensively for the control of agricultural and forest pests and also used as a public health insecticide for control of household insects, flies in animal houses, stored product insect pests, mosquito larvae and locusts.³ Generally, FN degrades in three ways: through hydrolysis,^{4,5} photolysis,^{1d} and microbial^{1(b),1(c),4} degradation.^{1f} Recent research has highlighted the role of metal ions in a biotic degradation processes for the OP ester and thioester pesticides.^{2(b-d),6-8}

The rates of hydrolysis in metal ion solutions, such as Cu^{2+} , Hg^{2+} and Ag^+ , were 20 - 3000 times faster than those of the uncatalyzed reactions.^{6,9} However, no direct experimental evidence has provided by the previous studies to show the interaction of OP pesticide with metal ion.^{6,9} It is postulated that the interaction of metal ions with atoms within $\text{O}=\text{P}$ or $\text{S}=\text{P}$ pesticides may enhance their hydrolytic degradation.

^{31}P NMR is sensitive to the structural environment, chemical shift values affording insight into factors such as electronegativity ($\text{P}=\text{O}$ vs. $\text{P}=\text{S}$) and $d(\pi)$ - $p(\pi)$ orbital overlap. ^{31}P NMR chemical shifts of OPs show upfield shifts when electronegative atoms or groups are attached,^{3,10} upfield shifts are also observed when transition metal ions are added,^{6,2(a),10} due to poorer electron density on the central P atom.^{1(e),7,10}

In order to find direct experimental evidence for the interaction of OP pesticides with Cu^{2+} ion, we carried out theoretical and experimental ^{31}P NMR studies. ESI-MS studies have also been carried out to obtain independent evidence on FA and FN- Cu^{2+} ion complexes. From the study of Cu^{2+} complex and

their fragmentation products, it may be possible to draw conclusion about the Cu^{2+} metal binding site and such information may give insight into the hydrolysis mechanism.

Experimental Section

Preparation of *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate (FN). Solution of dimethyl chlorothiophosphate (1 g, 6 mmol) in anhydrous ether (20 mL) was added dropwise to 3-methyl-4-nitrophenol (0.76 mL, 6 mmol) and triethylamine (0.83 mL, 5 mmol) in 20 mL anhydrous ether, and the mixture was stirred for 5 hours at 35 °C. The reaction mixture was evaporated under reduced pressure and the product was extracted with diethyl ether (100 mL). The diethyl ether extract was evaporated under reduced pressure. After evaporation of the solvent, the liquid product was isolated by silica gel column chromatography. The product was identified using ^1H and ^{13}C NMR spectroscopy (300 MHz Bruker). ^1H NMR (300 MHz, CDCl_3) δ 2.63 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 7.14 (m, 2H (aromatic)); ^{13}C NMR (75 MHz, CDCl_3) δ 20.96, 55.42, 55.49, 76.63, 77.48, 119.14, 124.65, 126.82, 136.46, 145.93, 153.52.

NMR study. The stock solutions of FN were prepared in acetone- d_6 and different concentrations of Cu^{2+} metal ion stock solutions were prepared in D_2O . For NMR analysis, 0.15 mL of metal ion stock solution was added to 0.45 mL of FN stock solution in NMR tube. The pH for stock solution mixtures was ~5.5 for Cu^{2+} metal ion. All ^{31}P NMR experiments on the FN were carried out in the absence or presence of Cu^{2+} metal ion using Bruker 300 MHz NMR spectrometer. In the presence of metal ion, ^{31}P NMR spectra were recorded after 5 minute of preparation. ^{31}P NMR chemical shifts (δ) are reported in parts per million (ppm) with respect to 85% H_3PO_4 used as an external chemical shift reference. The spectra were acquired using 90° pulses with 1000 - 1500 scans for ^{31}P nucleus.

Electrospray ionization mass spectrometry (ESI-MS). 0.10 mL of FN stock solution (0.01 M) prepared in dry acetone was

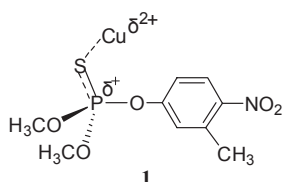
diluted with dry methanol to prepare 1.0×10^{-4} M solution. Cu (NO_3)₂ was dissolved in deuterium depleted water (DDW) for preparation of 5.0×10^{-5} M of Cu^{2+} metal ion solutions. Low micro molar FN solution in methanol and aqueous Cu^{2+} solutions were acidified to pH = 4 using 0.01M nitric acid solutions. Measurements were done immediately after the acidified solutions were mixed with various concentrations of aqueous Cu^{2+} . The mixture was sprayed with a flow rate of 6.0 $\mu\text{L}/\text{min}$ into a QSTAR XL QqTOF mass spectrometer (Applied Biosystems/MDS Sciex) employing a regular ESI source setup.

Results and Discussion

NMR study on FN in the absence and presence of Cu^{2+} ion.

NMR experiments were performed on FN with various concentrations of the catalytic Cu^{2+} ion. The ^{31}P NMR chemical shifts for OPs compounds show upfield shifts when more electronegative atoms bind to the central P atom.^{1,11} The systematic studies regarding the relative chemical shifts in the ^{31}P NMR spectra of OPs or related compounds are available in the literature.¹²⁻¹⁵ Table 1 shows the change in chemical shift ($\Delta\delta$) of ^{31}P as a function of concentration of Cu^{2+} ion. Change in chemical shifts ($\Delta\delta$) was calculated by subtracting the chemical shift values of ^{31}P in the presence of each metal ion from those in metal-free solution. Results in Table 1 clearly show the ^{31}P peak shifted upfield with increasing the concentration of Cu^{2+} , implying that Cu^{2+} bind to the S atom (1).

The upfield change in chemical shift could be attributed to interaction between each metal ion and FN. The chemical shift of the phosphorus atom bonded to the potential sulfur (S) atom is expected to be sensitive to the coordination of Cu^{2+} to the sulfur atom.



Theoretical ^{31}P NMR chemical shifts studies of the FN- Cu^{2+} complex. We have carried out some theoretical calculations to get structural information of the complexes between FN and copper cation and chemical shifts of phosphorous atom in the complexes. All the calculations have been carried out by using the Gaussian 98 program.¹⁶ All structures have been fully optimized at the density functional theory (DFT) of Becke's 3-parameter hybrid method using the correlation functional of Lee, Yang, and Parr (B3LYP)¹⁷ at 6-31G(d) level. Relativistic effective core potential of Los Alamos and Double- ζ basis sets (LANL2DZ)¹⁸ were employed for the copper cation. The calculations of absolute shielding constants have been performed using the Gauge-Independent Atomic Orbital (GIAO) perturbation method¹⁹ with HF/6-311+G(2d,p) and B3LYP/6-311+G(2d,p) levels. The optimized geometries of FN- Cu^{2+} and FN- Cu^{2+} -FN complexes in the gas phase are shown in Figure 1. The copper ion is bound to the sulfur atom tightly as expected by Pearson's

Table 1. ^{31}P NMR chemical shift of fenitrothion peaks in the absence and presence of various concentration of Cu^{2+} : $[\text{FN}] = 2.34 \times 10^{-2}$ M

$[\text{Cu}^{2+}] \times 10^2$ M	$[\text{Cu}^{2+}]/[\text{FN}]$	δ of ^{31}P	$\Delta\delta$
0.00	0.00	66.03	0.00
1.42	0.607	65.92	0.11
3.78	1.62	65.89	0.14
4.72	2.02	65.86	0.17
6.61	2.82	65.80	0.23

Table 2. GIAO calculation of absolute nuclear shielding of ^{31}P (in ppm) and net atomic charges of phosphorus and sulfur atoms

FN and Complexes	Charge (P, S) ^a	σ (δ) ^b	σ (δ) ^c	Exp(δ)($[\text{M}^{n+}]/[\text{FN}]$) ^d
FN	0.394, -0.170	261.9 (84.4)	205.3 (82.5)	66.03
FN-Cu	0.292, 0.253	262.8 (83.5)	206.6 (81.2)	65.80 (1:2.8)
FN-Cu-FN	0.583, -0.089	267.1 (79.2)	209.7 (78.1)	
H_3PO_4		346.3 (0)	287.8 (0)	0.0 (Standard)

^aHF/6-311+G(d) ^bHF/6-311+G(2d,p) ^cB3LYP/6-311+G(2d,p) ^dExperimental values.

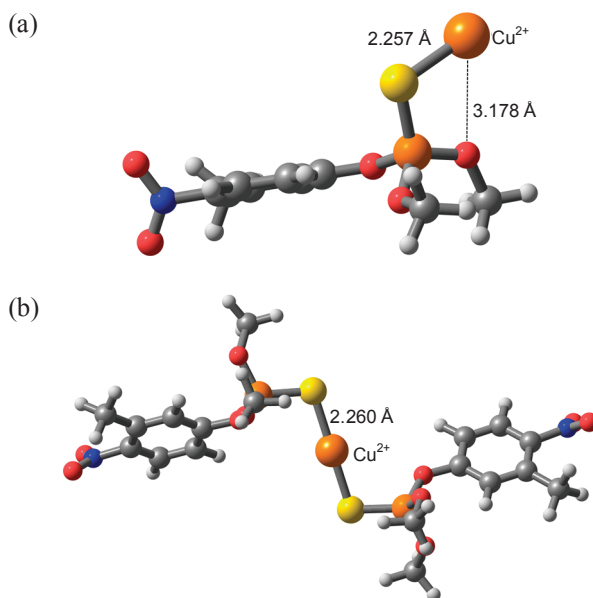


Figure 1. B3LYP/6-31G(d) structures of FN-Cu complex: (a) FN-Cu complex (b) FN-Cu-FN complex (C_2 symmetry).

hard and soft acid-base (HSAB) theory.²⁰ Absolute nuclear shielding constants, chemical shifts of the phosphorus and atomic net charges are shown in Table 2. The calculated results are in good agreement with the experimental ^{31}P NMR chemical shifts order, although the absolute shielding constants obtained by DFT method are much lower than those obtained by HF method. When compared with experimental result, ^{31}P NMR shift of complexes between FN and copper ions shows higher upfield change. Degree of the upfield shift of FN- Cu^{2+} -FN complex (2:1 complex) is larger than that of FN- Cu^{2+} complex (1:1 complex), and this coincides with the experimental finding that

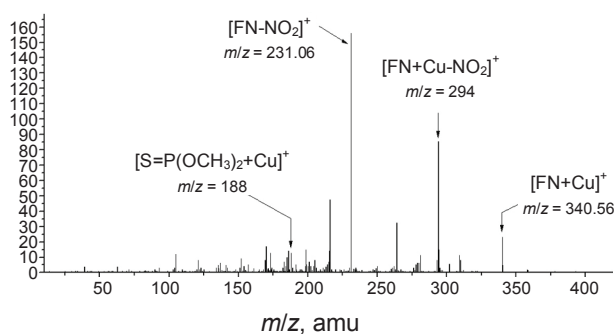


Figure 2. Positive ion ESI-MS spectrum of 50% aqueous methanol solution containing 5.0×10^{-5} M FN and 2.5×10^{-4} M Cu^{2+} ion.

the chemical shift of ^{31}P shifted upfield as the concentration of Cu^{2+} increased. In addition, the enhanced rate of degradation is attributed to the large positive charge developed on P atom in 2:1 complex (Table 3).

ESI-MS study in the FN and FN- Cu^{2+} complex. The FN pesticide was chosen to study possible interaction with transition metals. Possible interaction sites on FN are the free electron pairs on the S atom. The transition Cu^{2+} metal was chosen because of their possible interaction with sulfur containing ligand in solution. The Cu^{2+} ion has strong affinity to S atom²¹ in the compounds on the basis of HSAB theory.²¹ The ESI-MS and MS/MS data for the FN in methanol and FN with copper ions in 50% DDW-50% Methanol are summarized in Table 3. Protonated molecular ion peak at 278 (m/z) was attributed to $[\text{FN} + \text{H}]^+$. In order to further confirm the assignment of $[\text{FN} + \text{H}]^+$, MS/MS (CID) spectrum of $[\text{FN} + \text{H}]^+$ was measured and the spectrum shows ions at $m/z = 232$ and 125 for $[\text{FN} - \text{NO}_2 + \text{H}]^+$ and $[\text{S} = \text{P}(\text{OCH}_3)_2]^+$, respectively (Table 3). Copper has two isotopes (^{63}Cu and ^{65}Cu) with a natural abundance ratio of 69.09 : 30.91.²² MS spectrum of FN with Cu^{2+} ion shows two peaks corresponding to $([\text{FN} + \text{H}]^+)$ at $m/z = 278$, $([\text{FN} + \text{Cu}]^+)$, **2** at $m/z = \{340 (^{63}\text{Cu}), 342 (^{65}\text{Cu})\}$, and $([2\text{FN} + \text{Cu}]^+)$, **3** at $m/z = \{617 (^{63}\text{Cu}), 619 (^{65}\text{Cu})\}$ (Table 3). CID spectrum of $[\text{FN} + \text{Cu}]^+$ shows ions at $m/z = \{294 (^{63}\text{Cu}), 296 (^{65}\text{Cu})\}$, $\{188 (^{63}\text{Cu}), 190 (^{65}\text{Cu})\}$, and 231 for $([\text{FN} - \text{NO}_2 + \text{Cu}]^+)$, **4**, $([\text{S} = \text{P}(\text{OCH}_3)_2 + \text{Cu}]^+)$, **5**, and $[\text{FN} - \text{NO}_2]^+$, respectively (Figure 2 and Table 3). MS and CID spectrum results clearly support that the Cu^{2+} ion binds to the S atom as in the $[\text{FN} + \text{Cu}]^+$ MS peak and in the $[\text{FN} + \text{Cu} - \text{NO}_2]^+$ and $[\text{S} = \text{P}(\text{OCH}_3)_2 + \text{Cu}]^+$ CID peaks. The results obtained by the ESI-MS studies strongly support our conclusions drawn by NMR study.

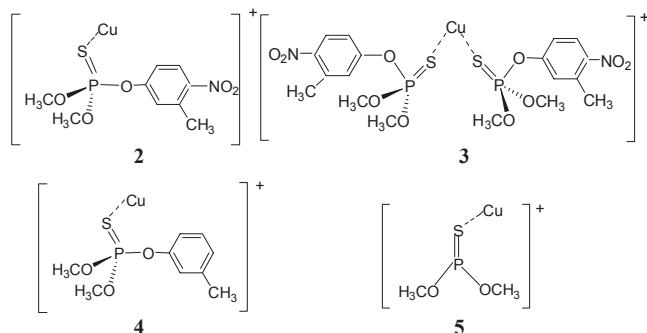


Table 3. ESI-MS and MS/MS data for the FN in methanol and FN with copper ions in 50% DDW-50% Methanol

Metal salt	Mode	Ions at m/z	Major species
No metal, H^+	MS	278 ^b	$[\text{FN} + \text{H}]^+$
	MS/MS	232 ^b	$[\text{FN} - \text{NO}_2 + \text{H}]^+$
		125 ^b	$[\text{S} = \text{P}(\text{OCH}_3)_2]^+$
CuNO_3^a	MS	617(^{63}Cu), 619(^{65}Cu)	$[2\text{FN} + \text{Cu}]^+$
		340(^{63}Cu), 342(^{65}Cu)	$[\text{FN} + \text{Cu}]^+$
		278	$[\text{FN} + \text{H}]^+$
	MS/MS	294(^{63}Cu), 296(^{65}Cu)	$[\text{FN} + \text{Cu} - \text{NO}_2]^+$
		188(^{63}Cu), 190(^{65}Cu)	$[\text{S} = \text{P}(\text{OCH}_3)_2 + \text{Cu}]^+$
		231	$[\text{FN} - \text{NO}_2]^+$

^aIn this table, the MS of ion containing copper is calculated in terms of ^{63}Cu and ^{65}Cu .

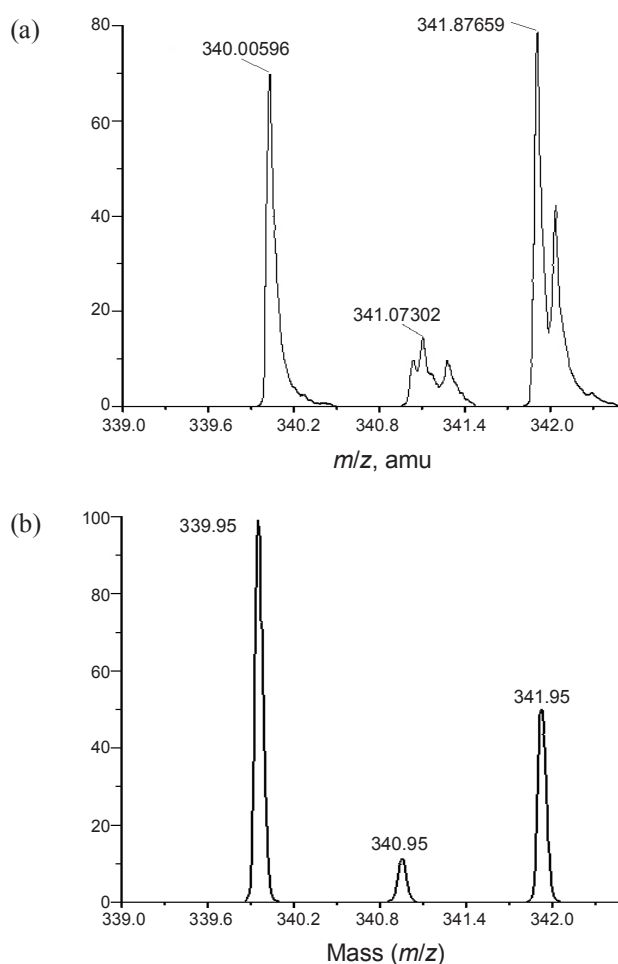


Figure 3. A representative figure showing the agreement between the experimental (a) and theoretical (b) peaks corresponding to $[\text{FN} + \text{Cu}]^+$ ion from ESI-MS of FN and Cu^{2+} ion.

Comparison of experimental versus theoretical MS Study. Verification of assignments of complex: Figure 3 shows the isotopes of copper, ^{63}Cu and ^{65}Cu . In order to confirm structural assignments, MS spectra for experimental (a) and the theoretical

(b) isotopic peaks corresponding to $([2\text{FN}+\text{Cu}]^+)$, **3** are compared. As can be seen from the example given in Figure 3, excellent agreement is observed between the theoretical and experimental spectrum. This type of comparison was done for all the metal complexes.

Conclusions

^{31}P NMR experiments and theoretical studies of FN and FN with Cu^{2+} ion gave valuable information for organophosphorus pesticide metal complexes. In addition, ESI-MS and low energy CID MS/MS experiments of FN with metal complexes combined with accurate mass measurement provided insight into the metal binding site and allowed unambiguous identification of fragments and hydrolysis products. Our results provided direct experimental and theoretical evidences for enhanced hydrolysis rates of organophosphorus pesticides in the presence of metal ions because of the feasible nucleophilic attacking to the electron deficient phosphorus atom by complexation of copper ion to the adjacent sulfur atom.

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