博士（地球環境科学）　モハメド　モウサ　イブラヒム　サイド

学位論文題名

CO_2 Hydration and Organophosphate Detoxification using Structural *Carbonic Anhydrase* and *Phosphatase* Models of Tripodal Ligand-based Zinc(Ⅱ) Complexes

（カルボニックアンヒドラーゼやホスファターゼ構造モデル、
トライポット型配位子からなる亜鉛化合物を用いた
二酸化炭素水和および有機リン系化合物無毒化）

学位論文内容の要旨

The present study was aimed at (i) the design and synthesis of tripodal ligand-based zinc(II) complexes, which structurally and functionally mimic the active site of zinc-containing enzymes such as *carbonic anhydrase* and *phosphatase* and (ii) their activities towards the hydration of CO_2 and the hydrolysis / detoxification of organophosphate nerve agents of various agricultural insecticides such as *parathion* and *paraoxon*. Consequently, these zinc(II) model complexes were used as bioinorganic catalysts to yield insight in the mechanism of action of these enzymes. The present work consists of two parts: (I) CO_2 hydration and calcification using zinc(II) model complexes, which structurally and functionally mimic the active site of *carbonic anhydrase* and *nacrein protein*; (II) Organophosphate hydrolysis / detoxification using zinc(II) model complexes structurally and functionally mimic the active site of *phosphohydrolases*.

To mimic the zinc(II)-coordination structure or the function of zinc(II) ion at the active site, we designed and investigated five different mononuclear zinc(II) model complexes **1**, **2**, **3**, **4**, and **5**, derived from tris(2-benzimidazolylmethyl)amine *L1*, N-{tris([2-methylbenzimidazole-2-yl)-ethyl]methyl]amino)-2-oxoethyl}iminodiacetic acid *L2*, tris(2-amino-ethyl)amine *L3*, *N,N',N''*-tris(2-benzylamino-ethyl)amine *L4*, and *N,N',N''*-tris(im-benzyl-L-histidylethylamino-ethyl)amine *L5*, respectively. Among the ligands, *L5* with three histidine side chains is featuring an environment analogous to the active center of the hydrolytic enzymes. These ligands and their zinc(II) model complexes were characterized by using various spectroscopic techniques (FT-IR, pH-potentiometric titrations, ^1H and ^{13}C NMR spectroscopies, and X-ray crystallographic studies). The activity of these zinc(II) model complexes towards the hydration of CO_2 and the hydrolysis / detoxification of organophosphates has been investigated by using IR, ^1H / ^{13}C NMR and UV-visible spectroscopies.

The results obtained during the course of study are highlighted as follows:

Part I: *chapter 2* describes CO_2 hydration using structural zinc enzyme model complexes mimicking the active site of *carbonic anhydrase*. The aqua zinc(II) complex $[L1Zn(H_2O)]^{2+}$ **1**$\cdot(PF_6)_2$, derived from tris(2-benzimidazolyl-methyl)amine *L1* has been designed, synthesized, and characterized. **1**$\cdot(PF_6)_2$ is the

structurally characterized monomeric aqua zinc(II) complex supported by imidazole functionalities and is therefore an excellent structural model for the active site of *carbonic anhydrase*. The *in vitro* simulation of CO_2 hydration has been investigated by using $\mathbf{1}\cdot(PF_6)_2$. The nucleophile $[L1Zn(OH)]^+$, which was produced by the deprotonation of $\mathbf{1}\cdot(PF_6)$, reacted with CO_2 gas to give a bicarbonate complex characterized by IR and ^{13}C NMR spectroscopies. The molecular structure of $[L1Zn(NCS)]_2[(Zn(NCS)_4$ **1''**, containing coordinated NCS^- anion as a model for carbonic anhydrase inhibitor has been also determined and adopted slightly distorted tetrahedral ZnN_4 coordination geometry with the equatorial positions occupied by three benzimidazole nitrogen atoms and the apical position by NCS^- anion; *Chapter 3* describes a novel structural zinc enzyme model for CO_2 hydration and calcification. A new ligand: *N*-{tris([2-[1-methylbenzimidazole-2-yl)ethyl]methyl]amino)-2-oxoethyl}iminodiacetic acid has been synthesized. Ligand *L2* was used to prepare a new zinc(II) complex **2** as a promising model for the active site of the nacreous protein in mollusk shells. The CO_2 hydration and calcification have been studied in the light of the influence of the pK_a value of the coordinated water molecule and the carboxylate groups.

Part II: *Chapter 5* describes phosphoester hydrolysis using structural *phosphatase* models of tren based zinc(II) complexes and X-ray crystal structures of $[Zn(tren)(H_2O)](ClO_4)_2$, $[Zn(tren)(Cl)](BPh_4)$,and $[Zn(tren)(BNPP)](ClO_4)$ (tren = tris(2-aminoethyl)-amine, $BNPP^-$ = bis(*p*-nitrophenyl)phosphate). New tris(2-aminoethyl)amine *L3* based ligands, namely *N,N',N''*-tris(2-benzylamino-ethyl)amine *L4* and *N,N',N''*-tris(im-benzyl-L-histidylethyl-amino-ethyl)amine *L5* have been synthesized and characterized. Complexation studies on Zn^{2+} complexes **3**, **4**, and **5** derived from *L3*, *L4*, and *L5* showed that the presence of benzyl and benzyl-histidyl moieties attached to the tripodal ligand L3 side arms decrease the pK_a of the Zn-bound water molecule: 10.72 for **3**, 9.61 for **4**, and 7.43 for **5**, respectively. The zinc complex of **5** was a more active catalyst for the hydrolysis of $BNPP^-$, O,O-diethyl-*p*-nitrophenyl thiophosphate (parathion), and tris(*p*-nitrophenyl)phosphate (TNPP) compared to **3** and **4**. In the case of **3** and **4**, the pH-dependence of their observed pseudo-first-order rate constants k_{obs} showed sigmoidal pH-rate profile, while **5** gave bell-shape curve with a maximum rate constant of around 1.05×10^{-5} s^{-1} in the case of $BNPP^-$ and 1.36×10^{-5} s^{-1} in the case of parathion at pH 9.0. The pH dependence of k_{obs} indicated that the Zn—bound hydroxo species is responsible for the hydrolytic activity. The crystal structures of $[L3Zn(H_2O)](ClO_4)_2$ **3**, $[L3Zn(Cl)]$-(BPh_4) **3'**, and $[L3Zn(BNPP)]ClO_4$ **3''** have been determined and showed trigonal-bipyramidal configurations around the central Zn. The zinc complexes of **3** and **3''** served as structural models for the binding mode of coordinated water as well as coordinated substrates in the active site of zinc enzyme; *chapter 6* describes solution-solid state structural studies of *carbonic anhydrase* inspired *phosphatase* model: the catalyzed hydrolysis of parathion. The tripodal ligand tris(2-benzimidazolyl-methyl)amine *L1* was used for the preparation of three novel zinc(II) complexes, $[L1Zn(OH)]^+$ $\mathbf{1'}\cdot ClO_4$, $[L1Zn(H_2O)]^{2+}$ $\mathbf{1}\cdot(BNPP)_2$, and $[L1Zn(DETP)]ClO_4$ **1'''** ($DETP^-$ = O,O-diethyl thiophosphate). The molecular structures of $\mathbf{1}\cdot(BNPP)_2$ and **1'''** adopt slightly distorted tetrahedral ZnN_3O coordination geometries with the equatorial positions occupied by three benzimidazole nitrogen atoms and the apical position by oxygen atom from H_2O molecule in complex $\mathbf{1}\cdot(BNPP)_2$ and from $DETP^-$ anion in complex **1'''**. In complex $\mathbf{1}\cdot(BNPP)_2$, $BNPP^-$ is not coordinated to the zinc(II) ion. Instead, the oxygen atoms of $BNPP^-$ anions participate in two hydrogen bonds to the coordinated water. Apparently, these hydrogen bonds prevented the $BNPP^-$ from approaching the zinc ion, therby inhibiting the hydrolytic reaction. Solution studies (pH and ^1H NMR titrations) of the ligand *L1*

with Zn(II) ions gave detailed information about the structure of the resulting zinc complexes and the evidence for the existence of the zinc-bound hydroxo species. In parallel with the solid state structures of **1**·$(BNPP)_2$ and **1'''**, the hydrolytic activity by using $L1Zn^{2+}$ as potential catalyst towards the hydrolysis of parathion and $BNPP^-$ was also examined.

学位論文審査の要旨

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学位論文題名

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（カルボニックアンヒドラーゼやホスファターゼ構造モデル、
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目的は，(1)carbonic anhydrase(CA)や phosphatase(PH)の活性中心の構造・機能の生体模倣によって，三脚型配位子からなる亜鉛酵素モデル化合物の分子設計と合成，(2)二酸化炭素水和や有機リン酸系化合物，殺虫剤の paraoxon や parathion の加水分解，無毒化の発現である．当学位論文は２つの部分(I)CA 活性中心を模倣した亜鉛モデル化合物を用いた二酸化炭素の水和や(II)PH のそれを模倣した有機リン酸系化合物の加水分解・無毒化から成り立っている．

亜鉛酵素モデル化合物 **1**, **2**, **3**, **4**, **5** はそれぞれ配位子，tris(2-benzimidazolylmethyl) amine **L1**, N-{tris([2-methylbenzimidazole-2-yl)-ethyl]methyl]amino)-2-oxoethyl} iminodiacetic acid **L2**, tris(2-amino-ethyl)amine **L3**, N,N′,N″-tris(2-benzylamino-ethyl)amine **L4**, N,N′,N″-tris(im-benzyl-L-histidylethylamino-ethyl)amine **L5** から合成された．

これらの化合物の組成・構造が，^{1}H ・ ^{13}C NMR，元素分析，pH 滴定，亜鉛滴定，FT-IR および X 線結晶解析によって明らかにされた．当亜鉛化合物を用いた水和反応や加水分解反応は ^{1}H ・ ^{13}C NMR および UV-visible 吸収スペクトル測定から研究された．

本論文は７章からなり，第１章では CA や亜鉛酵素モデル化合物を用いた二酸化炭素水和反応について集録・言及した．第２章では CA の活性中心を模倣した亜鉛酵素モデル化合物[L1Zn(H_2O)]$^{2+}$ **1** を用いて CO_2 水和を再現した．そ

の際，**L1** の構成単位である benzimidazolyl の役割によって構造的・機能的な面から CA に類似したモデル化合物となった．CA による酵素反応に対する陰イオン阻害剤モデルとして$[L1Zn(NCS)]_2[(Zn(NCS)_4$ の分子構造を結晶解析から明らかにした．Zn(II)は benzimidazolyl の窒素原子３個が equatorial の位置に，NCS^-が apical の位置に配位して４面体の立体配置を示していることを明らかにした．更に，第３章ではCO_2水和と石灰化の２つの反応が促進されるべき，新規な配位子 *N*-{tris([2-[1-methylbenzimidazole-2-yl)ethyl]methyl]amino)-2-oxoethyl}iminodiacetic acid を分子設計・合成を行った．溶液中での当配位子や，亜鉛化合物のpK_aおよび錯形成定数を pH 滴定から決定した．然しながら，水和や石灰化の反応に関する研究は，その亜鉛化合物が溶解する溶媒がなくて成功しなかった．

第４章から第７章までは PH の亜鉛モデル化合物として$[L3Zn(H_2O)]^{+2}$ **3**，$[L4Zn(H_2O)]^{+2}$ **4**，$[L5Zn(H_2O)]^{+2}$ **5** を用いて，基質としての有機リン系化合物 bis(*p*-nitrophenyl)phosphate($BNPP^-$) や O,O-diethyl-*p*-nitrophenyl thiophosphate (parathion)の加水分解反応に関して記述されている．上記の３種類の配位子と各々の亜鉛モデル化合物の 33%メタノール(v/v)水溶液を対象に pH 滴定とその曲線の再現によって各配位子や亜鉛に配位している水分子の酸解離定数pK_aおよび鉛モデル化合物の安定度定数k_{st}の pH 依存性を決定した．更に ^1H NMR 測定によって亜鉛滴定を行い，亜鉛と配位子から 1:1 の亜鉛化合物のイオンが溶液中に存在することおよび化合物と配位子との間の化学交換の速さについて言及している．基質としての$BNPP^-$の場合，擬一次速度定数$k_{obs}=1\times10^{-6}\sim1\times10^{-5}\ s^{-1}$，parathion の場合$k_{obs}=1\times10^{-3}\sim1.5\times10^{-2}\ s^{-1}$の pH 依存性を示した．尚，$k_{obs}$は加水分解生成物の *p*-nitrophenolate の吸収ピーク(~400nm)強度の時間依存性から決定された．$BNPP^-$や parathion の両者に対して，**3** と **4** の場合k_{obs}の pH 依存性を sigmoidal そして **5** の場合のそれは bell-shape の示した．いずれの曲線の編曲点は亜鉛モデル化合物の配位水のpK_aに対応していた．**5** の配位水の低いpK_a(=7.4)であることおよび$-OH^-$やOH_2が亜鉛に配位している２種類の **5** が共存していることの為に，他の **3**，**4** と比べて加水分解反応をより促進させたと考えられる．**3** のは最小の値を示した理由は，$BNPP^-$が亜鉛に配位することによって加水分解反応が進行しにくくなることをその配位構造の結晶解析から明らかにした．

以上，本研究で得られた知見は，有機リン系化合物の殺虫剤加水分解による無毒化の指針を与えるもので，環境問題のひとつに貢献すると期待される．

審査委員一同はこれらの成果を評価し，大学院課程における取得単位なども併せて申請者が博士（地球環境科学）の学位を受けるに充分な資格を有するものと判定した．