

tic H), 7.57-7.88 (m, 2H, aromatic H); Mass (m/e) 223 (M⁺, 8), 221 (53), 193 (21), 177 (57), 149 (42), 105 (36), 77 (16), 73 (100); IR (ν_{max}) 1771 cm⁻¹.

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Comparable Structural Stabilities of Penta- and Hexa-coordinate Zn(II) in a Simple Model System of the Active Site of Carboxypeptidase A

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Ab initio studies of simple model systems for the carboxypeptidase A active site indicate that penta- and hexa-coordinate Zn(II) complexes have comparable structural stabilities. These facile coordination structures can be responsible for the catalytic role. Although the hexa-coordinate Zn(II) complex is more stable in enthalpy than the penta-coordinate Zn(II) complex, the entropy effect makes the latter as stable as or slightly more stable in free energy than the former.

Introduction

Zinc chemistry is an area of great importance because its occurrence in biology is second only to that of iron among transition elements. More than 80 enzymes containing zinc have been reported¹. For example, zinc is essential in biological functions of enzymes such as carboxypeptidase, alcohol dehydrogenase, carbonic anhydrase, β-lactamase and phospholipase. Bovine carboxypeptidase A (CPA) catalyzes the cleavage of the peptide bond of C-terminal amino acids. The hydrolytic mechanism of this enzyme has attracted much interest as a prototype of mechanistic study of metalloprotease, but is still of much controversy²⁻⁵. It is known that the zinc ion in the metalloprotease plays a central role in the catalytic mechanism from the fact that the complete loss of catalytic activity is observed in the case of apoenzyme⁶. X-ray study reported that the zinc ion in CPA is coordinated

by two histidines (His-69 and His-198), a bidentate glutamate (Glu-72), and water molecules⁷. The distances from Zn²⁺ to the nearest water oxygen atoms are 2.05 and 3.23 Å (Table 1). Then, the number of ligating water molecules can be regarded as either one or two, because the second nearest water oxygen is in between first and second hydration shell water oxygens⁸. In complexation of CPA with inhibitors, the coordination number of Zn²⁺ varies between 5 and 6^{9,10}. Previously, we studied the hydration structures¹¹ and ammoniation structures¹² of Zn, finding that the coordination number of the hydration slightly favors 6, while that of the ammoniation favors 4. Here, we study very simple model systems around Zn²⁺ mimicking the active site of CPA. Since the Zn²⁺-ligand complexation in CPA is directly related to the enzymatic mechanism of CPA, we focus on the coordination structure of Zn²⁺.

Model Systems and Computational Methods

Ab initio calculations were performed on a series of model complexes. The model complexes comprise a zinc ion ligated by a formate ion (HCOO^-), amino residues, and 0-2 water molecules. We have undertaken *ab initio* calculations of four model complexes of $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})_n]^+$ ($n=0-2$). One model complex of $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)]^+$ (1) is the case of the free enzyme without water. Two model complexes of $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})]^+$ (2) and $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})_2]^+$ (3) are the cases when one water molecule is added to 1 without and with coordination to Zn^{2+} , respectively. The fourth model complex of $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})_2]^+$ (4) is the case when two water molecules are added to 1 with coordination to Zn^{2+} . Then, the coordination numbers of Zn^{2+} in 1, 2, 3 and 4 are 4, 4, 5 and 6, respectively.

For the study of Zn-containing enzymes ammonia was often used as model for imidazole in histidine residues for computational feasibility in compensation for accuracy. Although there is some difference in binding energetics between ammine and imidazole interacting with Zn^{2+} , the relative binding energetics between ammonia-Zn(II) complexes (1-4) would not seriously differ from those between the corresponding imidazole-Zn(II) complexes because of the cancellation effect on the relative binding energetics in that the two imidazole groups are replaced by two ammonia molecules for all the four systems. It is thus expected that the ammonia- Zn^{2+} complex may represent the successive binding energetics for added water molecules reasonably well^{13,14}.

For the four model systems 1-4, we have performed Hartree-Fock (HF) calculations using the *8s6p2d* basis set¹¹ for Zn and the Dunning¹⁵ *DZ+d* basis sets for all the other atoms. The exponents of a set of five *d* functions for O, N and C are 0.85, 0.80 and 0.75, respectively. Full geometry optimization was done using a suite of Gaussian 92 program¹⁶, and the four model complexes were confirmed to be the minimum energy structures with vibrational frequency analysis. The basis set superposition error correction (BSSEC) was done with full basis sets¹⁷. The importance of the BSSEC is found in various references^{18,19}. For the purpose of incorporating the electron correlation, Møller-Plesset second order (MP2) calculations were performed at the HF optimized geometries. The thermodynamic quantities were calculated using the vibrational frequencies and binding energies¹⁹

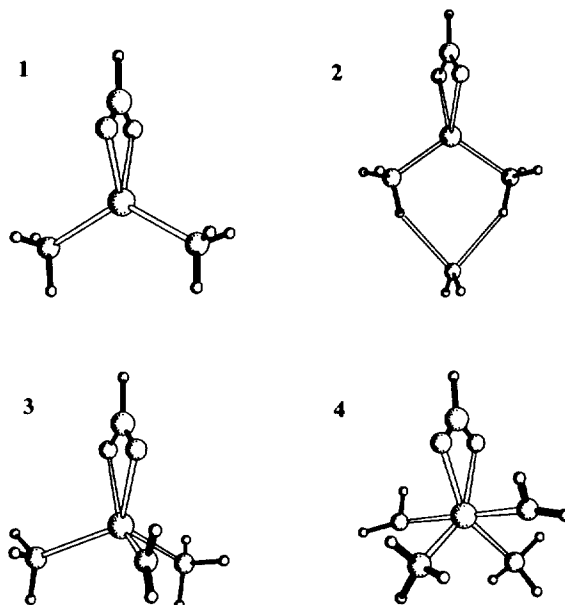


Figure 1. Structures of $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)]^+$ (1), $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})]^+$ (2), $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})_2]^+$ (3), and $[\text{Zn}(\text{NH}_3)_2(\text{HCOO}^-)(\text{H}_2\text{O})_2]^+$ (4).

Results and Discussion

The predicted distances between Zn^{2+} and the coordinating atoms in the model complexes are listed in Table 1 and compared with the x-ray data of CPA. The individual and average Zn-ligand distances from the model complexes of 3 and 4 are in reasonable agreement with the x-ray results in consideration of the complexity of enzymatic environments.

Table 1 also lists the complexation energies of the model complexes: the binding energies (BE_{hf} , BE_c and BE_{zp}) at 0 K, enthalpy change (ΔH_r), and Gibbs free energy change (ΔG_r), where *r* denotes room temperature (298 K) at 1 atm. The BE_{hf} is the HF-predicted binding energy with BSSEC. The BE_c is the MP2 binding energy (calculated at the HF optimized geometry) with BSSEC. The BE_{zp} is the sum of the BE_c and the zero-point energy change (ΔZPE) obtained from the HF harmonic vibrational frequencies. For strongly bound complexes such as ion-ligand systems with strong

Table 1. Distances between Zn^{2+} and coordinating atoms (Å) in complexes 1-4 and the thermodynamic energies for complexation (kcal/mol)^a

Complex	(sym)	$d_{\text{Zn-N}}$	$d_{\text{Zn-O}} (f)$	$d_{\text{Zn-O}} (w)$	d_{avg}	BF_{hf}	BE_c	BE_{zp}	ΔH_r	ΔG_r
1	(C_{2v})	2.09, 2.09	2.03, 2.03		2.06	-463.7	-450.3	-442.4	-444.3	-418.1
2	(C_{2v})	2.08, 2.08	2.03, 2.03	3.81	2.06	-503.2	-507.7	-497.2	-499.8	-463.8
3 ^b	(C_1)	2.11, 2.11	2.04, 2.15	2.17	2.12	-509.6	-513.0	-503.0	-505.3	-470.2
4 ^b	(C_2)	2.14, 2.14	2.13, 2.13	2.28, 2.28	2.14	-521.7	-525.5	-513.4	-516.2	-470.7
CPA ^c		2.07, 2.13	2.18, 2.31	2.05, 3.23	2.15					

^a $d_{\text{Zn-O}} (f)$ and $d_{\text{Zn-O}} (w)$ are the distances between Zn and HCOO^- oxygens and between Zn and water oxygen, respectively; d_{avg} is the average distance from Zn to all coordinating atoms. See the text for other notations. The HF energies of Zn^{2+} , NH_3 , HCOO^- and H_2O are -1776.78040, -56.19848, -188.23411 and -76.03461 hartrees, and their MP2 energies are -1777.32955, -56.37911, -188.75385 and -76.23165 hartrees, respectively. ^b Entropy correction of RT ln 2 (=0.4 kcal/mol) was made for the chiral molecules: Pitzer, K. B. *J. Chem. Phys.* 1939, 7, 251. ^c Ref. 7.

electrostatic interaction, the thermodynamic quantities based on the binding energies and vibrational frequencies are relatively in good agreement with experimental data¹⁹⁻²⁵.

From the comparison of the free energy changes at 298 K and 1 atm (ΔG_r), **3** is much more stabilized than **2** (by 6.4 kcal/mol). Namely, one water molecule added to **1** tends to ligate the Zn^{2+} ion. When one water molecule is added to **3**, the enthalpy of **4** is much lower than that of **3** by 10.9 kcal/mol. But, the free energy of **4** is only 0.5 kcal/mol lower than that of **3**, which indicates that the structure with the coordination number 5 is as stable as that with the coordination number 6. Accordingly, the molecular stability toward the coordination number 5 arises from the entropy effect. If the free energy loss (2.1 kcal/mol) for isolation of one water molecule from bulk water is taken into account²⁶, the structure with the coordination number of 5 becomes slightly more stable in aqueous solution than that with the coordination number 6. Thus, our calculation shows that the entropy effect makes the penta-coordinate Zn(II) complex as stable as or slightly more stable than the hexa-coordinate Zn(II). That is **3** and **4** have comparable stability. This is likely to be in line with Lipscomb *et al.*'s proposal that a substrate cannot completely exclude water from the zinc site for the duration of substrate hydrolysis⁷.

Concluding Remarks

We performed *ab initio* studies of model complexes for the carboxypeptidase A active site. Although the hexa-coordinate Zn(II) complex is more stable in enthalpy than the penta-coordinate Zn(II) complex, the entropy effect makes the latter as stable as or slightly more stable than the former. The comparable stabilities between the penta-coordinate structures suggest that possible variation of the coordination number in Zn-containing enzymes may help substrate or inhibitors easily access to or recede from the coordination sites of Zn^{2+} , which is likely to be one of the central roles of Zn^{2+} in CPA. The issue that our results can be directly applicable to the CPA active site is not clear because our model systems are too simple. Thus, our calculations provide only a possibility for the operation of metal-substrate interactions in the enzymatic reaction. Nevertheless, our results suggest that the facile structural change in the Zn-ligand coordination may be responsible for a certain enzymatic reaction.

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