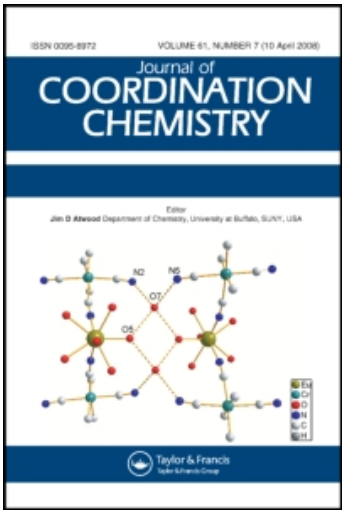

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Excess Zinc Ions Induce the Inhibition of Carboxypeptidase A Activity by the Conformational Change

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EXCESS ZINC IONS INDUCE THE INHIBITION OF CARBOXYPEPTIDASE A ACTIVITY BY THE CONFORMATIONAL CHANGE

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Abstract: The mechanism for inhibition of enzyme activity by excess zinc ions has been studied by kinetic and equilibrium dialysis method at pH 8.2. Excess zinc ions were competitive inhibitor for both peptide and ester substrates. This behavior is believed to arise by the excess zinc ions fixing the enzyme in a conformation to which substrate cannot bind.

Keywords: Excess zinc ions, carboxypeptidase

INTRODUCTION

Carboxypeptidase A ([(CPD)Zn]) is a zinc enzyme catalyzing the hydrolysis of the carbonyl terminal residue from peptide or ester substrates by cleavage of the peptide or ester bond. Crystallographic studies indicate that in the enzyme the side-chain oxygen of Tyr-248 is about 17 Å away from the Zn atom and that the phenolic oxygen moves 12 Å toward active site as substrates bind.[1]

Arsanilazotyrosine-248 carboxypeptidase A ([(Azo-CPD)Zn]) is a derivative of carboxypeptidase A with selectively modified chromophoric arsanilazotyrosine-248 residue, exhibiting no significant effect on its catalytic activities.[2,3] At pH 8.2, the arsanilazotyrosine residue forms an intramolecular complex with the zinc ion in the active site of the

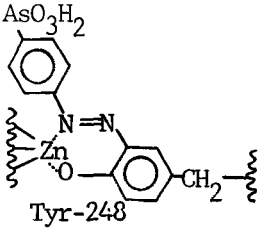

FIGURE 1

enzyme (FIGURE. 1) and gives rise to a 510 nm peak.[2,3] When excess zinc ions bind to [(Azo-CPD)Zn], the characteristic red color, which arises from the intramolecular complex of the arsanilazotyrosine-248 residue with the active site zinc atom of the enzyme, change to yellow as the arsanilazotyrosine residue is released from the coordination sphere of the zinc ion in the active site.

$$[(\text{Azo-CPD})\text{Zn}]\text{Zn}^{2+}(\text{yellow}) \rightleftharpoons [(\text{Azo-CPD})\text{Zn}](\text{red}) + \text{Zn}^{2+} \quad (1)$$

This behavior indicates that a conformational change of protein occurs in [(Azo-CPD)Zn].

Recently, we have found that excess zinc ions inhibit the enzyme activity of arsanilazotyrosine248-carboxypeptidase A.[4] A more detailed study of the relationship between the color change and the inhibition of the enzyme by excess zinc ions was studied[5] and is reported here.

MATERIALS AND METHODS

[(CPD)Zn](Bovine Pancreas) was obtained from Sigma. [(Azo-CPD)Zn] was prepared by the method of Johansen et al.[2] Carbobenzoxy-glycyl-L-phenylalanine(CBZ-Gly-Phe), benzoyl-glycyl-L-phenylalanine (Bz-Gly-Phe), and benzoyl-glycyl-L-phenyllactate (Bz-Gly-OPhe) were used as substrates.

RESULTS AND DISCUSSION

The enzyme activity of [(CPD)Zn] and [(Azo-CPD)Zn] in the presence of excess zinc ions was measured for peptide (CBZ-Gly-Phe, Bz-Gly-Phe) and ester substrates (Bz-Gly-OPhe). In all substrates, intersection points of straight lines in Lineweaver-Burks plots were on 1/v axis, and the Cornish-Borden plots of the same data had apparently parallel lines. This behavior indicates that zinc ion is a competitive inhibitor for both peptide and

ester substrates with [(Azo-CPD)Zn] and [(CPD)Zn]. Table I contains the value of K_i and mode of the inhibition by zinc ions of [(CPD)Zn] and [(Azo-CPD)Zn]. The K_i values for excess zinc ions with all substrates in [(Azo-CPD)Zn] and [(CPD)Zn] are the same (K_i=5.2-2.6x10^{-5} M).

Apparent dissociation constant ($K_{d(Azo-CPD)}$) of excess zinc ions from [(Azo-CPD)Zn]Zn^{2+} in Eq. 1 which is obtained from absorption change at 510 nm was 3.2x10^{-5} M. And $K_{d(Azo-CPD)}$ is very similar to the K_i of [(Azo-CPD)Zn]. The apparent constant ($K_{d(CPD)}$) for dissociation of excess zinc ions from carboxypeptidase A([(CPD)Zn]Zn^{2+} $\rightleftharpoons$ [(CPD)Zn] + Zn^{2+}) obtained by the equilibrium dialysis at pH 8.2 was 2.4x10^{-5} M and very close to K_i values above.

The apparent dissociation constant and inhibition constant of excess zinc ions obtained by the inhibition of the enzyme activity, spectrophotmetric, and the equilibrium dialysis method of both native [(CPD)Zn] and [(Azo-CPD)Zn] were almost the same. This coincidence between the apparent dissociation constants and inhibition constants indicates that the zinc binding to the enzyme directly relates to the inhibition of enzyme activity and the conformational change of protein by excess zinc ions. This behavior is believed to arise by the excess zinc ions binding to the enzyme and fixing the enzyme in a conformation to which the substrates cannot bind.

TABLE I. Inhibition of Carboxypeptidase A and Arsanilazotyrosine-248 Carboxypeptidase A Catalyzed Hydrolysis of Peptide and Ester Substrates by Excess Zinc Ions and Dissociation Constants of Zinc Ions for Carboxypeptidase A and Arsanilazotyrosine-248 Carboxypeptidase A obtained by Equilibrium Dialysis and Spectrophotometric Method at pH 8.2.

	[(CPD)Zn]		[(Azo-CPD)Zn]	
	$K_i \pm SD$ (μM)	mode	$K_i \pm SD$ (μM)	mode
(I)enzyme activity				
Bz-Gly-Phe	52±18	competitive	35±10	competitive
CBZ-Gly-Phe	52±16	competitive	30±6	competitive
Bz-Gly-OPhe	26±3	competitive	31±5	competitive
	$K_{d(CPD)} \pm SD$ (μM)		$K_{d(Azo-CPD)} \pm SD$ (μM)	
(II)spectrophotometric	20±5		32±5 27±6 (10°C)	
(III)equilibrium dialysis	24±5 (10°C) 6.3±2 (10°C, pH 9.0)			

All experiments were performed in 0.05 M Tris-HCl buffer containing 0.5 M NaCl.

REFERENCES

1. W.N. Lipscomb, J.A. Hartsuck, G.N. Reeke, F.A. Quincho, P.H. Betnge, M.L. Ludwing, T.A. Steiz, H. Muirhead, and J.C. Coppola, Brookhaven Symp, Biol., 21, 24 (1968).
2. J.T. Johansen, and B.L.Vallee, Proc. Natl. Acad. Sci. U.S.A., 70, 2006 (1973).
3. L.W. Harrison, D.S. Auld, and B.L. Vallee, Proc. Natl. Acad. Sci. U.S.A., 72, 4356 (1975).
4. J. Hirose, M. Noji, Y. Kidani, and R.G. Wilkins, Biochemistry, 24, 3495 (1985)
5. J. Hirose, S. Ando, and Y. Kidani, Biochemistry, 26, 6561 (1987)