Metallocarboxypeptidases: Stability Constants and Enzymatic Characteristics*

JOSEPH E. COLEMAN† AND BERT L. VALLEE

From the Biophysics Research Laboratory of the Department of Medicine, Harvard Medical School, and the Peter Bent Brigham Hospital, Boston, Massachusetts

(Received for publication, March 12, 1961)

In a previous communication (1), we have described some of the enzymatic and physicochemical properties of a series of metallo-carboxypeptidases prepared from the metal-free apoenzyme of bovine carboxypeptidase A. This paper presents the stability constants and enzymatic activities of a series of metallo-carboxypeptidases produced when 1 g atom of zinc in the native enzyme is replaced by 1 g atom of manganese, cobalt, nickel, copper, cadmium, or mercury. Significantly, the substitution of the various metals for zinc in carboxypeptidase induces marked changes in relative activities and specificity of the enzyme. The order and magnitude of the stability constants of this series of metallo-carboxypeptidases are in accord with the hypothesis that a sulfur and a nitrogen atom of the apoenzyme bind these metal ions to constitute the active center (2-4). The release of two H⁺ ions when a metal atom is bound to apocarboxypeptidase constitutes further experimental support for this hypothesis, since the apparent pK values of the ionizing groups are consistent with those to be expected of a sulfhydryl and a nitrogenous group of amino acid residues (5).

EXPERIMENTAL PROCEDURE

Beef Pancreas Carboxypeptidase [(CPD)Zn]—Four times recrystallized zinc carboxypeptidase was prepared from beef pancreas acetic powder by the method of Allan et al. The solution of the final crystals in 1.0 M NaCl-0.1 M Tris buffer, pH 7.5, was homogeneous in the ultracentrifuge and by moving boundary electrophoresis when examined in 0.3 ionic strength LiCl buffers of pH 6.6 to 10.5. The proteolytic coefficient, C, of this preparation was 25 to 30 at pH 7.5, 25°, and 6 to 7 at pH 7.5, 0°. The esterase activity, expressed as a zero order rate constant, k (see below), was 1.15 × 10⁷ M⁻¹ H⁺ per minute per mg of N at pH 7.5, 25°. The zinc to protein ratio was 1950 µg per g of protein or 1.08 g atom per mole, assuming a molecular weight of 34,300 for the protein (6, 7).

Zinc-free Apocarboxypeptidase (CPD)—Zinc-free apocarboxypeptidase was prepared by dialyzing the native enzyme against 1.10-phenanthroline. Precautions against contamination by metal ions were taken, as previously described (1, 8). The preparations used in these experiments contained between 26 and 39 µg of zinc per g of protein, or 1.3% and 2% of the original zinc content, and had 2% of the original activity.

Enzymatic Activity—Peptidase activity was determined as described elsewhere (1) with carbobenzoxyglycyl-β-phenyllactate and carbobenzyglycyl-L-tryptophan, and benzoylglycyl-L-phenylalanine as substrates (Mann Chemical Company). Activity is expressed as an apparent proteolytic coefficient, C, as previously defined and employed (1, 9, 10). The assays were carried out at 0° in 0.02 M sodium Veronal buffer containing 0.1 M NaCl, pH 7.5; C was calculated from the linear portion of the first order reaction plots observed when hydrolysis did not exceed 15%.

Esterase Activity was as described (11) with hippuryl-dl-β-phenyllactate as the substrate. The activity was measured by titration with 0.1 M NaOH of the hydrogen ions released on hydrolysis with a pH Stat titrator (Radiometer, Copenhagen) and recorder (Ole Dich, Copenhagen). Assays were performed at 25° with 5 ml of 0.01 M hippuryl-dl-β-phenyllactate in 0.1 M NaCl. 0.005 M Tris buffer, at pH 7.5. Activities are expressed as zero order velocity constants, k, with units of 10⁻⁴ M⁻¹ H⁺ per minute per mg of N.

Metal Analyses—Zinc, cadmium, mercury, copper, manganese, nickel, cobalt, and lead were determined by chemical methods which are specific for each (12-16).

Protein Concentrations were measured either by 10% trichloroacetic acid precipitations followed by drying at 104° (17), or from absorbancy at 278 m (18). The results of the two procedures were in excellent agreement. The Beckman model DU spectrophotometer was used throughout, and pH was measured with a Leeds and Northrup pH indicator equipped with a general purpose external glass electrode.

Preparation of Zinc, Cadmium, Mercury, Lead, Manganese, Cobalt, Nickel, and Copper Carboxypeptidase—Standard solutions of zinc, cadmium, mercuric, manganous, cobaltous, nickelous, cupric, and lead chlorides were prepared by dissolving the spectrographically pure metals (Johnson Matthey Company, Ltd.) in dilute, metal-free HCl. The solutions were diluted with metal-free buffer to result in the desired pH and molarity, to be

† Gift of Dr. H. Neurath.
‡ All buffers, unless otherwise indicated, were 1.0 M NaCl and 0.05 M Tris.

* This work was supported by a grant in aid from the National Institutes of Health of the Department of Health, Education and Welfare, No. H3117(C1).
† Post-Doctoral Fellow of the National Institutes of Health of the Department of Health, Education and Welfare.


‡ Kindly supplied by the Lilly Research Laboratories.
dialyzed against the isotopically labeled enzymes for exchange experiments, or against the zinc-free apoenzyme to prepare a given metallocarboxypeptidase. The metal solutions used for the exchange and equilibrium experiments described below were labeled by the addition of tracer amounts of isotope to the standard solutions.

**RESULTS**

**Stability Constants—**Manganese, cobalt, nickel, and copper carboxypeptidase undergo significant dissociation under the experimental conditions here used for equilibrium dialysis, 1 M NaCl-0.05 M Tris, pH 8.0, 4°C. Hence, the apparent stability constants, \(K_\text{app} = ([\text{CPD}]\text{Me})/([\text{CPD}])(\text{Me}^{++})\), can be calculated from measurements of protein, either by trichloroacetic acid precipitation or by absorbancy at 278 nm, and from measurement of the respective metal ion, by isotopic or microchemical means. This “direct method” has been described (1) for measurement of the stability constant of the cobalt enzyme, the prototype for this procedure.

Once the stability constant for one of these metallocarboxypeptidases is obtained in this manner, that for any other can be determined by allowing a second metal ion to compete for the enzymatic binding site, to result (at equilibrium) in a mixture of two metalloenzymes. By use of this “competition method,” the following relationship holds:

\[
K_\text{app} = \frac{([\text{CPD}]\text{Me})([\text{CPD}]\text{Me}_0)}{([\text{CPD}]\text{Me}_0)([\text{CPD}]\text{Me})} \cdot K_{\text{Me}_0}
\]

in which \(\text{Me}_0\) represents the metal for which the stability constant is known and \(\text{Me}_0\) that for which it is unknown.

Apocarboxypeptidase forms a 1:1 complex with each metal, as determined by isotopic and microchemical analyses. The apparent stability constants, \(K_\text{app}\), for the resultant metallocarboxypeptidases follow the order Zn > Cd > Hg > Ni > Co > Mn > Cu. (Table I, Columns A and B).

Because the solubility characteristics of the enzyme required the presence of 1 M NaCl and 0.05 M Tris, the \(K_\text{app}\), deviates from the true stability constant, \(K_{\text{corr}}\), due to the competition between these anions and the apoenzyme for the metal ions. It is, therefore, necessary to calculate the concentration of free metal ions by correcting for that moiety, bound to chloride and Tris, by which the effective concentration of free metal ions in solution is diminished. The constants for ammonia may be expected to approximate those for Tris, and the correction is significant for all the ions but Mn (19).

\[
K_{\text{corr}} = \frac{([\text{CPD}]\text{Me})([\text{CPD}]\text{Me}_0)}{([\text{CPD}]\text{Me}_0)([\text{CPD}]\text{Me})} \cdot K_{\text{Me}_0}
\]

The log of the corrected stability constants, \(K_{\text{corr}}\), are shown in Table I, Column E, and follow the order Zn > Cd > Hg > Ni > Co > Mn > Cu. (Table I, Columns A and B). Table I gives similar data for glycine and ethylenediamine as examples of nitrogen-oxygen or nitrogen-nitrogen ligands. Finally, Columns E and F give similar data for glycine and ethylenediamine as examples of nitrogen-oxygen (N-O) and nitrogen-nitrogen (N-N) ligands, respectively. The \(K_{\text{corr}}\) for the metallocarboxypeptidases, and those of the nitrogen-sulfur series are closely correlated (Columns C and D); in both instances, the complexes with Zn and Cd are more stable than those of Co and Ni. In contrast, there is no correlation between the constants of the metallocarboxypeptidases in Column C and those of the nitrogen-oxygen or nitrogen-nitrogen series in Columns E and F.

**Table I**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Dissociation constant (apparent)</th>
<th>Log (K_{\text{corr}}) (apparent)</th>
<th>Log (K_{\text{corr}}) (CF, Tris)</th>
<th>Log (K_1) (N-S)</th>
<th>Log (K_1) (N-O)</th>
<th>Log (K_1) (N-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>2.5 x 10^-4</td>
<td>5.6</td>
<td>5.6</td>
<td>4.1</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Co</td>
<td>1.5 x 10^-4</td>
<td>5.8</td>
<td>7.0</td>
<td>7.7</td>
<td>5.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Ni</td>
<td>2.0 x 10^-4</td>
<td>5.7</td>
<td>8.2</td>
<td>9.9</td>
<td>6.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Zn</td>
<td>7.4 x 10^-8</td>
<td>5.1</td>
<td>10.6</td>
<td>10.2</td>
<td>8.6</td>
<td>10.7</td>
</tr>
<tr>
<td>Cd</td>
<td>4.7 x 10^-4</td>
<td>8.3</td>
<td>10.5</td>
<td>10.2</td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Hg</td>
<td>1.2 x 10^-4</td>
<td>7.9</td>
<td>10.8</td>
<td>11.0</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>2.0 x 10^-7</td>
<td>6.7</td>
<td>21.0</td>
<td>22.0</td>
<td>10.3</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Each contained 1 g atom of metal per mole of protein. The oxidation state of the copper is not known.

Determined in 1 M NaCl-0.05 M Tris, pH 8.0, 4°C.

These values for 2-mercaptoethylamine are not on record; those given are for cysteine (19).

The reasons for the unavailability of constants for the cupric complexes have been detailed (20, 21).
Metallocarboxypeptidases

When carboxyamino-glycyl-L-tryptophan or benzoylglycyl-L-phenylalanine are used as the substrates, the activities of the cobalt enzyme are approximately equal to those of the zinc enzyme in contrast to the rates observed with carboxyamino-glycyl-L-phenylalanine (Table II). The activities of nickel carboxypeptidase are much lower, whereas those of manganese carboxypeptidase are the same with all three substrates. Copper, lead, cadmium, and mercury carboxypeptidase do not hydrolyze any of these peptides. Thus, the particular metal atom which occupies the single active site markedly and differentially affects the rates at which carboxypeptidase hydrolyzes peptide substrates.

Carboxypeptidase, like other peptidase, hydrolyzes esters of which is hippuryl-dl-β-phenyllactate. With the exception of copper carboxypeptidase, which hydrolyzes neither peptides nor this ester, the metallocarboxypeptidases incorporating the metals of the first transition series studied here also attack this ester substrate. However, mercury, cadmium, and lead carboxypeptidase exhibit esterase activities while being completely inactive toward peptides; cadmium and mercury carboxypeptidase, in fact, are more active than is the zinc enzyme (Table II).

The complete loss of peptidase activity observed on replacing zinc by mercury, cadmium, or lead made it important to determine whether these metals occupy the same or different sites on the enzyme as zinc and hence to discern whether the curtailment of the dual specificity for peptides and esters involves binding of these metal ions to the same or different sites of the molecule. To test this possibility, [(CPD)Zn] was exposed to 1 x 10^{-4} M stable cadmium ions. Bound radioactive zinc and proteolytic activity were determined simultaneously at various times (Fig. 2). The loss of peptidase activity correlates directly with the displacement of zinc, both being complete at 96 hours, when all zinc is replaced by cadmium. In a separate experiment, [(CPD)Zn], the native enzyme, was exposed to Cd^{2+}, and esterase activity was measured (Fig. 2). As zinc is displaced, protein-bound Cd^{2+} and esterase activity rise concurrently to reach 1 g atom of cadmium per mole of enzyme: the

Table II

<table>
<thead>
<tr>
<th>(CPD)Me^a</th>
<th>Peptidase activity^b</th>
<th>Esterase activity^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Carboxyamino-</td>
<td>Carboxyamino-</td>
</tr>
<tr>
<td></td>
<td>glycyl-L-tryptophan</td>
<td>glycyl-L-tryptophan</td>
</tr>
<tr>
<td>[(CPD)Zn]</td>
<td>7.5</td>
<td>2.8</td>
</tr>
<tr>
<td>[(CPD)Co]</td>
<td>12.0</td>
<td>2.7</td>
</tr>
<tr>
<td>[(CPD)Ni]</td>
<td>8.0</td>
<td>2.0</td>
</tr>
<tr>
<td>[(CPD)Mn]</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>[(CPD)Cu]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[(CPD)Hg]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[(CPD)Cd]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[(CPD)Pb]</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

^a Me, 1 g atom per mole of apoenzyme.

^b Assays were carried out at pH 7.5, 25°C, with 0.02 M substrate in 0.02 M sodium Veronal, containing 0.1 M NaCl.

^c Assays were carried out at pH 7.5, 25°C, with 0.01 M hippuryl-dl-β-phenyllactate in 0.005 M Tris, containing 0.1 M NaCl. The coefficient of variation for these measurements is 4%.
The mole fractions of [(CPD)Cd] and [(CPD)Zn] present constituted zinc enzymes interact with reagents specific for mercapto groups, whereas one -SH group is readily titrated in to consist of a zinc atom bound to a sulfur and nitrogen atom. Thus, the localization of the zinc mercaptide involved in activity is limited to one particular residue, cysteine, in the amino acid sequence of the apoenzyme. Previous evidence also indicated that cobaltous ions, like zinc, bind to a sulfur group. These two ions are bound to the same site, as proven by exchange experiments (1). Hence, the specific activity of the cobalt enzyme, higher than that of the zinc enzyme, could not be attributed to the creation of an additional, active site, contrary to other reports (23). The spectral changes accompanying the formation of cobalt carboxypeptidase show the formation of a mercapptide linkage with apocarboxypeptidase. Cobalt carboxypeptidase exhibits a distinctive red color with an absorption maximum at 530 m\textmu and an extinction coefficient of 150 (1). The shift in the absorption maximum from 512 m\textmu in the hydrated cobaltous ion to 530 m\textmu in [(CPD)Co], together with the increase in the extinction coefficient from 10 to 150, suggest binding to sulfur (24).

Similar binding of nickel, manganese, mercury, and cadmium to a sulfur atom is indicated by the relative order of the stability constants for the respective metallocarboxypeptidases which follow that expected for a sulfur containing ligand. The sulfur-nitrogen nature of the bidentate binding site is implied by the magnitude of the stability constants and by the release of two H\textsuperscript{+} ions on combination of the apoenzyme with Zn\textsuperscript{2+} ions. The participation of a nitrogen atom as the second donor atom in binding zinc to apocarboxypeptidase was deduced from a number of considerations (4), which apply to this series of metallo-carboxypeptidases.

**DISCUSSION**

These data confirm and extend past observations and hypotheses indicating the active center of native carboxypeptidase to consist of a zinc atom bound to a sulfur and nitrogen atom (2, 4). It may be recalled that neither the native nor the reconstituted zinc enzymes interact with reagents specific for mercapto groups, whereas one —SH group is readily titrated in the metal-free apocarboxypeptidase; furthermore, restoration of activity through the addition of metal ions is prevented when the —SH group is blocked. Thus, the localization of the zinc mercaptide involved in activity is limited to one particular residue, cysteine, in the amino acid sequence of the apoenzyme.
The ranking order of stability constants for the complexes of a series of metal ions with simple ligands is found to be characteristic of its constituent donor atoms. Thus, when nitrogen and oxygen function in this capacity, the ranking order for the stability constants of metal complexes interest here follows the sequence $\text{Hg}^{++} > \text{Cu}^{++} > \text{Ni}^{++} > \text{Co}^{++} > \text{Zn}^{++} > \text{Cd}^{++} > \text{Fe}^{++} > \text{Mn}^{++}$ (25-27), as is apparent from the examples shown in Table I, Columns E and F. It should be noted that the stability of the nickel and cobalt complexes is equal to or greater than that of the zinc and cadmium complexes.

The substitution of sulfur for one of the donor atoms, to yield a sulfur-nitrogen ligand, brings about marked changes in this sequence, which becomes $\text{Hg}^{++} > \text{Cd}^{++} > \text{Zn}^{++} > \text{Ni}^{++} > \text{Co}^{++} > \text{Fe}^{++} > \text{Mn}^{++}$, copper being indeterminate because of the oxidation of the mercapto group (20, 21) (Table I, Column D). The complexes of cadmium and zine are now distinctly stabilized over those of cobalt and nickel, the characteristic feature of the sulfur ligand series (25, 26, 28).

Hence, through the determination of the stability constants of such a series of metal complexes, the donor atoms of the metal-binding site of an organic ligand may be determined. If the same considerations can be shown to apply for metalloenzymes, a method for the identification of the ligand groups of the corresponding apoenzymes is at hand.

The sequence of the stability constants of the metallocarboxy- peptidases studied here is in the order $\text{Hg}^{++} > \text{Cd}^{++} > \text{Zn}^{++} > \text{Ni}^{++} > \text{Co}^{++} > \text{Fe}^{++} > \text{Mn}^{++}$, analogous to that of the sulfur-containing ligands (Table I, Columns C and D). It has been suggested (3) that the correlation both of the sequence and of the magnitudes of the constants implies that nitrogen and sulfur donor groups are the common denominator for metal binding of apocarboxypeptidase and the bidentate ligands in Table I, Column D. Hence, it would follow that all of the metals of this series interact with the sulfur-nitrogen site of apocarboxypeptidase, in the manner of site-specific reagents.

These interpretations are supported by evidence obtained independently from measurements of the release of $\text{H}^+$ ions from apocarboxypeptidase on addition of $\text{Zn}^{++}$ ions at pH 7. The ionizations of the two donor groups are well separated and can, therefore, be determined quite accurately to correspond to $\text{pK}$ values of 7.7 and 9.1, respectively, under the experimental conditions here used (Fig. 1); these conditions were not designed to evaluate the contribution of ionization of neighboring groups, the dielectric constant of the medium, its ionic strength, electrostatic effects, or hydrogen bonding, all well known to modify the acid-base properties of amino acids when incorporated into proteins (29-32).

The acid-base properties of $\alpha$-amino and imidazolium groups are most pertinent to the assignment of the $\text{pK}_A$ of 7.7 to a group of carboxypeptidase. This value is certainly compatible with those published for $\alpha$-amino groups, varying from 7.7 to 7.9 in peptides (30) or from 7.45 to 7.9 in proteins (33, 34). Aspartagine contributes the single $\alpha$-amino group of carboxypeptidase (35).

An imidazole group, however, is not eliminated readily from consideration. The $\text{pK}_A$ of the imidazolium group of histidine is thought to be between 5.6 and 6.8 in peptides (30) and from titration data has been assigned values from 6.4 to 7.0 in proteins (33, 34, 35-38). The upper range of ionization of imidazoles has even been thought to extend to pH 8, although this interpretation of the titration data does not take account of the contribution of $\alpha$-amino groups (31). The possibility of a much lower $\text{pK}_A$ has been mentioned for the especially reactive histidine group of ribonuclease (39, 40). An $\alpha$-amino group seems to us the one most likely to be involved in metal binding of carboxypeptidase; precise identification, however, must await the results of analytical work now in progress.

The $\text{pK}_A$ of the second group, 9.1, could correspond to that of an $\alpha$-SH group, in accord with the values obtained for $\alpha$-SH groups of peptides (28) or, less likely, to that of an $\epsilon$-amino group (38). This problem is somewhat simplified since the participation of the sole mercapto group of carboxypeptidase in zinc binding has been demonstrated analytically (2).

Thus, although there is a single binding site of apocarboxypeptidase, its complexation of different metals imparts different specific activities to carboxypeptidase towards a given substrate. It has been demonstrated previously and is here confirmed that carboxypeptidases formed by complexation with metals of the first transition series, including manganese, nickel, cobalt, and zinc, attack substrates containing the peptide nitrogen or the oxygen of the ester bond (1, 4). Remarkably, enzymatic hydrolysis can be restricted to esters: complexes of apocarboxypeptidase with the group II B elements cadmium and mercury or with lead exhibit esterase activity only, but do not hydrolyze the synthetic peptides tested thus far (Table II). The experiments in which the zinc atom of carboxypeptidase is exchanged for either mercury or cadmium (Figs. 2 or 3) demonstrate moreover that the same site of the enzyme is involved in binding all of these, as demonstrated already for cobalt (1); hence, the simultaneous increase in esterase activity and the abolition of peptidase activity accompanying this replacement cannot be attributed to complex formation of these metals with a different site of the apoenzyme. This curtailment of the dual enzymatic specificity of native carboxypeptidase is particularly striking on comparing the rates of hydrolysis of benzoylglycyl-$\epsilon$-phenylalanine and hippuryl-$\beta$-$\beta$-phenylactate, structurally differing only by the substitution of the ester for the peptide bond (Table II).

Thus, the metal atom known to function in substrate binding (41) also plays a role in the determination of enzymatic specificity. The coordination of the respective nitrogen or oxygen atoms of the substrates with the metal atom of the active enzymatic center will likely prove significant in detailing the mechanism of carboxypeptidase action. Many analogous aspects of the catalytic action of metals in peptidases have been discussed (42, 43).

The role of metal ions in the action of carboxypeptidase is apparent in yet another manner. Under standard conditions of assay, the relative order of catalytic efficiencies of different metallocarboxy- peptidases varies as a function of the primary structure of the synthetic peptide substrate. Thus, the ranking order $\text{Co} > \text{Ni} > \text{Zn} > \text{Mn}$ observed previously for carbobenzoxylglycyl-$\epsilon$-phenylalanine (1) is confirmed here and is preserved for a wide range of substrate concentrations, ionic strengths, and other conditions of assay (4), but it is inverted to $\text{Zn} > \text{Co} > \text{Ni} > \text{Mn}$ for both carbobenzoxylglycyl-$\epsilon$-tyro- phan and benzoylglycyl-$\epsilon$-phenylalanine and also the ester substrate, hippuryl-$\beta$-phenylactate.

The exact chemical mechanism responsible for these striking changes in catalytic activity, brought about by the different metals in conjunction with the same apoenzyme operating on
different substrates, remains to be determined, although the groups of the protein responsible for binding the metal are becoming apparent.8

SUMMARY

Apocarboxypeptidase forms enzymatically active complexes with a series of metal ions. The stability constants are in the order $\text{Hg}^{++} > \text{Cd}^{++} > \text{Zn}^{++} > \text{Ni}^{++} > \text{Co}^{++} > \text{Mn}^{++}$. Both the order and the magnitudes of the constants correlate closely to those found to be characteristic of nitrogen-sulfur ligands, implying that in carboxypeptidase a nitrogen-sulfur site binds metal ions to result in enzymatic activity. This interpretation is supported by titrations showing two metal binding groups with $pK$ values of 7.7 and 9.1, respectively, compatible with published values for $\alpha$-amino and $\text{--SH}$ groups. A zinc mercapto at the active center of carboxypeptidase has been demonstrated (2). Manganese, cobalt, nickel, and zinc carboxypeptidases hydrolyze both peptides and an ester substrate, hippuryl-dl-P-phenyllactate. The ranking order of peptidase activity includes one sulfur, one nitrogen, and one zinc atom. Suggests that the active center of native carbosypeptidase A is one of these, as shown previously for cobalt (1). The evidence demonstrates that the same site of the enzyme is involved in binding all of these, as shown previously for cobalt (1). The evidence suggests that the active center of native carboxypeptidase A includes one sulfur, one nitrogen, and one zinc atom.

REFERENCES


Metallo-carboxypeptidases: Stability Constants and Enzymatic Characteristics
Joseph E. Coleman and Bert L. Vallee


Access the most updated version of this article at
http://www.jbc.org/content/236/8/2244.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/236/8/2244.citation.full.html#ref-list-1