Amino Acid Composition and Effect of pH on the Kinetic Parameters of Renal Dipeptidase*

ALEX M. RENÉ AND BENEDICT J. CAMPBELL
From the Department of Biochemistry, University of Missouri, Columbia, Missouri 65201

SUMMARY

The procedure for the purification of renal dipeptidase from particulate kidney cortex fractions has been modified to eliminate a time-consuming washing process and a chromatographic step which frequently led to loss in activity. The over-all purification resulted in the isolation of 5.8 mg of purified peptidase from 1.5 kg of kidney cortex and produced an enzyme approximately 500 times more active than source material.

Physical and chemical properties such as sedimentation characteristics, electrophoretic behavior, and zinc content were compared with those reported for earlier peptidase preparations. The amino acid composition of the enzyme based on a molecular weight of 47,200 indicates a protein that contains approximately 414 amino acid residues.

A study of the effect of pH upon the kinetic parameters of renal dipeptidase indicates that the NH₂-terminal amino group of the peptide substrate dissociates a proton during the catalytic process. Furthermore, it is suggested that a dissociation of pK 8.5 which takes place from the enzyme-substrate complex results from the loss of a proton from a water molecule coordinated to zinc at the active center of the enzyme.

Earlier work in these laboratories led to the isolation of highly purified renal dipeptidase (1). The purified enzyme exhibited homogeneity during ultracentrifugation and in acrylamide gel electrophoresis experiments. The molecular weight of the peptidase was reported to be 47,200, and zinc analyses showed that the enzyme contains 1 g atom of zinc per mole of protein.

Glycyldehydrophenylalanine, the assay substrate employed in the purification procedures, was found to undergo copper-activated hydrolysis, and this reaction was investigated as a possible model system for peptidase-catalyzed hydrolysis (2, 3). From the data obtained, it was concluded that a 1:1 copper-dipeptide complex is formed in which the copper ion is coordinated to the amino-terminal nitrogen, the amide nitrogen, the carboxyl oxygen of the peptide, and to a water molecule. The data further support the suggestion that the loss of a proton from the coordinated water molecule must take place in order for copper-activated hydrolysis of unsaturated dipeptides to occur. It was also shown that the rate of copper-activated hydrolysis is directly related to the association constant of the labile complex.

In the present investigation the previously reported isolation procedure (1) has been modified to eliminate a time-consuming washing operation and a chromatographic procedure that frequently resulted in loss of activity. The new procedure made possible the preparation of sufficient renal dipeptidase for determination of the amino acid composition of the enzyme. The effect of pH on the kinetic parameters of the peptidase was studied to obtain results for comparison with the data obtained in the metal-activated model system.

EXPERIMENTAL PROCEDURE

Purification of Renal Dipeptidase—The enzyme was separated from fresh hog kidney cortex by means of a modification of previously reported procedures (1, 4). In the earlier methods water-soluble proteins were removed from the kidney particulate material by washing for at least 7 days through Hyflo Super-Cel on Whatman No. 4 paper. This procedure, which involved many changes of filtering material to insure an adequate rate of filtration in the cold, resulted in considerable loss of time. However, the particulate fraction must be free from soluble protein at this stage since later procedures do not completely remove the soluble protein contaminants. In the present method this washing step was carried out as follows. The cold acetone precipitate (4) was collected by centrifuging at 0° and 8000 × g for 40 min. The supernatant was tested for the presence of protein by the addition of 10% trichloracetic acid. Resuspension with 0.066 M phosphate buffer at pH 7 followed by precipitation with 1 M HCl was repeated until the supernatant no longer gave a positive test for trichlor-
acetic acid-precipitable protein. Solubilization of the final pH 5 precipitate was carried out with 1-butanol as previously described (4).

Further purification of the solubilized peptidase was performed according to earlier methods (1), including ammonium sulfate fractionation, carboxymethyl cellulose chromatography, and Sephadex G-200 gel filtration. In the present procedure, however, a Sephadex G 150 elution step was substituted for the previously employed diethylaminoethyl cellulose chromatographic step. The new gel filtration procedure was carried out as follows. A Sephadex G-150 column, 2.5 cm × 90 cm, was equilibrated with 0.02 M Tris-HCl buffer containing 10⁻⁴ M ZnCl₂ at pH 8.0. In a typical experiment, 10 mg of protein in 2 ml of the Tris-HCl buffer was applied to the column. Elution with the same buffer was carried out at a flow rate of 25 ml per hour. A volume of 5 ml of effluent per tube was collected. The absorbance of each tube at 280 μ and 260 μ was measured, and enzyme activity was assayed as described below.

Renal Dipeptidase Assays—Soluble peptidase fractions were assayed by measuring the fall in optical density at 275 μ of solutions of various glycyglycyldehydrophenylalanine concentrations (2). The temperature of reaction was controlled, usually at 35°, by means of a temperature-controlled jacket through which water was circulated from a Brinkmann-Haake ultra thermostat at 35° for 6-hour intervals until a dry constant weight was obtained. Further purification of the solubilized peptidase was performed according to earlier methods (1), including ammonium sulfate fractionation, carboxymethyl cellulose chromatography, and Sephadex G-200 gel filtration. In the present procedure, however, a Sephadex G 150 elution step was substituted for the previously employed diethylaminoethyl cellulose chromatographic step. The new gel filtration procedure was carried out as follows. A Sephadex G-150 column, 2.5 cm × 90 cm, was equilibrated with 0.02 M Tris-HCl buffer containing 10⁻⁴ M ZnCl₂ at pH 8.0. In a typical experiment, 10 mg of protein in 2 ml of the Tris-HCl buffer was applied to the column. Elution with the same buffer was carried out at a flow rate of 25 ml per hour. A volume of 5 ml of effluent per tube was collected. The absorbance of each tube at 280 μ and 260 μ was measured, and enzyme activity was assayed as described below.

Renal Dipeptidase Assays—Soluble peptidase fractions were assayed by measuring the fall in optical density at 275 μ of solutions of various glycyglycyldehydrophenylalanine concentrations (2). The temperature of reaction was controlled, usually at 35°, by means of a temperature-controlled jacket through which water was circulated from a Brinkmann-Haake ultra thermostat at 35° for 6-hour intervals until a dry constant weight was obtained. Further purification of the solubilized peptidase was performed according to earlier methods (1), including ammonium sulfate fractionation, carboxymethyl cellulose chromatography, and Sephadex G-200 gel filtration. In the present procedure, however, a Sephadex G 150 elution step was substituted for the previously employed diethylaminoethyl cellulose chromatographic step. The new gel filtration procedure was carried out as follows. A Sephadex G-150 column, 2.5 cm × 90 cm, was equilibrated with 0.02 M Tris-HCl buffer containing 10⁻⁴ M ZnCl₂ at pH 8.0. In a typical experiment, 10 mg of protein in 2 ml of the Tris-HCl buffer was applied to the column. Elution with the same buffer was carried out at a flow rate of 25 ml per hour. A volume of 5 ml of effluent per tube was collected. The absorbance of each tube at 280 μ and 260 μ was measured, and enzyme activity was assayed as described below.

Characterization of Renal Dipeptidase—The purified peptidase was examined for gross heterogeneity by analytical ultracentrifugation in the Spinco model E ultracentrifuge at 59,780 rpm in 0.02 M Tris-HCl buffer at pH 8.0. Polyacrylamide-gel electrophoresis experiments were performed using the disc electrophoresis method described by Davis (6). Zinc analyses were carried out by atomic absorption spectroscopy in the following manner. Enzyme samples were ashed in platinum crucibles. The ashed samples were dissolved in dilute hydrochloric acid (Mallinckrodt analytical reagent grade) and quantitatively transferred to volumetric flasks. Determinations of zinc were made on a Perkin-Elmer atomic absorption spectrophotometer model 903. The zinc contents of the enzyme samples were obtained by comparing the absorption measurements to a standard curve constructed from measurements of standard solutions of zinc chloride prepared from zinc metal (Fisher certified reagent, Lot No. 543900). The moisture content of lyophilized enzyme samples was determined by the method of Lowry et al. (5). The initial rate of reaction was obtained from the slope of a plot of optical density versus time. The initial rate of reaction was obtained from the slope of a plot of optical density versus time. The initial rate of reaction was obtained from the slope of a plot of optical density versus time. The initial rate of reaction was obtained from the slope of a plot of optical density versus time.

Amino Acid Analyses—Samples of purified renal dipeptidase (1.44 mg) were hydrolyzed in glass-redistilled 6 M HCl under nitrogen in sealed tubes at 110° for 24, 48, 72, and 96 hours. The tubes were opened and their contents were dried in a vacuum desicator over sodium hydroxide at room temperature. The residue was freed of remaining HCl by redissolution in water and subsequent drying in the same manner. Redissolution and drying were repeated three times. The final residue was dissolved in 0.2 M sodium citrate buffer at pH 2.2, filtered through scintillated glass, and the final volume made up to 5 ml with the same buffer. Aliquots of 2 ml of the protein hydrolysate were analyzed according to the procedure of Moore, Spackman, and Stein (8) with a Beckman-Spinco model 120 amino acid analyzer. The values for serine, threonine, and tyrosine were corrected for decomposition during hydrolysis by extrapolating the 24-, 48-, and 72-hour values in a linear fashion to zero time of hydrolysis. Also the amounts of glutamic acid was calculated from the ammonia content of the 24-, 48-, and 72-hour hydrolysates after extrapolation to zero time of hydrolysis. Valine and isoleucine were incompletely released at short times of hydrolysis, and for that reason their values were calculated from the 96-hour hydrolysates.

In the chromatograms where methionine sulfoxide was detected, the methionine value was corrected for the value of the methionine sulfoxide. Identification and quantification of the peaks were made in comparison with several standard runs made on the same analyzer with the standard amino acid calibration mixture, Beckman-Spinco type I Lot CM 115, immediately before sample analysis. Tryptophan was determined in a sample of unhydrolyzed protein by the method of Bencze and Schmid (9) with the value determined for tyrosine on the amino acid analyzer in the final calculation. Determination of Kinetic Parameters as Function of pH—Ratios of glycine-glycyldehydrophenylalanine hydrolysis were measured as described above. The concentration of renal dipeptidase used in these experiments was 0.893 μg per ml. Substrate concentrations employed were 8.00 × 10⁻⁴ M, 1.50 × 10⁻⁴ M, 3.75 × 10⁻⁴ M, and 1.50 × 10⁻⁴ M. Reactions in the pH range from 5.2 to 7.4 were in 2 M piperazine-N₂N₂-bis (2-ethane sulfonic acid)-monocodium monohydrate. Reactions in the pH range from 7.2 to 9.3 were in 2 M piperazine-N₂N₂-bis (2-ethane sulfonic acid)-monocodium monohydrate. Reactions in the pH range from 7.2 to 9.3 were in 2 M piperazine-N₂N₂-bis (2-ethane sulfonic acid)-monocodium monohydrate. Reactions in the pH range from 7.2 to 9.3 were in 2 M piperazine-N₂N₂-bis (2-ethane sulfonic acid)-monocodium monohydrate.

Materials—Glycine-glycyldehydrophenylalanine was synthesized by methods previously described (2). The product melted at 255-257° and had a molecular extinction coefficient of 1.53 × 10⁴ at 275 μ.
RESULTS AND DISCUSSION

Removal of soluble proteins from the particulate peptidase fraction by precipitation at pH 5 and washing with 0.066 M phosphate buffer provided a much less time-consuming method for obtaining an insoluble fraction of adequate activity for further purification. The isolation of the enzyme by gel filtration on Sephadex G-150 is shown in Fig. 1. Under these conditions the activity was found as a shoulder of the second protein peak eluted from the column. The first and last peaks were devoid of peptidase activity, and the fact that these peaks exhibit higher absorbance at 260 μ suggests that they contain nucleotide material. A summary of the solubilization and purification of renal dipeptidase is shown in Table I. From 1.5 kg of kidney cortex, 132 g of protein homogenate were obtained, and the complete purification from this homogenate yielded 5.8 mg of peptidase. The over-all purification produced an enzyme approximately 500 times more active than source material.

The schlieren photographs of the high speed sedimentation analysis of renal dipeptidase exhibited only one symmetrical peak after 59 minutes at 59,780 rpm, confirming a previous report of the sedimentation characteristics of similar preparations (1). Fig. 2 shows the results of acrylamide-gel electrophoresis of solubilized peptidase fractions. These results indicate a gradual elimination of protein bands until the last step in the purification is reached. This final product appears monophoretic. Three different determinations of the zinc content of the enzyme by atomic absorption spectroscopy gave values of 1.40, 1.39, and 1.40 μg of zinc per mg of protein. Calculation of the mole ratio of zinc to enzyme with a molecular weight of 47,200 confirms that 1 mole of peptidase contains 1 g atom of zinc. The lyophilized enzyme was found to contain 4.69 ± 1.07% of its weight as moisture. The relatively low precision of the moisture determination resulted from the small quantities of enzyme available for this analysis. Analysis for nitrogen gave a value of 15.76 ± 0.07%. Measurement of the absorption spectrum of 0.135 mg of renal dipeptidase in 1 ml of 0.002 M Tris-HCl buffer at pH 8.0 showed a minimum absorption at 251 μ and a maximum at 280 μ; no unusual absorption properties were exhibited. The ratio A260:A280 was 2.25. The extinction coefficient (ε280) determined with salt free protein and corrected for moisture content was 8.96. Results of the amino acid analysis of the enzyme are presented in Table II. The percentage of recovery of nitrogen in the amino acid analysis compared with the Ballentine method (7) was 100.82%. The amino acid composition of the enzyme based on the data on Table II, assuming a molecular weight of 47,200, indicates a protein that contains approximately 414 amino acid residues.

Prior to using the purified peptidase in quantitative rate studies the effect of enzyme concentration upon the rate of hydrolysis was established. In Fig. 3 it is shown that the relationship between enzyme concentration and enzyme activity is linear over the range of enzyme concentrations employed. A time study of the peptidase-catalyzed hydrolysis of glycyldelhy-
TABLE II

Amino acid composition of renal dipeptidase

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid residues</th>
<th>Nitrogen Residues per molecule</th>
<th>Assumed residues per molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.81</td>
<td>1.70</td>
<td>28.65</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.91</td>
<td>0.88</td>
<td>9.93</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.63</td>
<td>2.03</td>
<td>17.08</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>9.51</td>
<td>1.15</td>
<td>38.83</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.72</td>
<td>0.65</td>
<td>21.92</td>
</tr>
<tr>
<td>Serine</td>
<td>5.17</td>
<td>0.85</td>
<td>28.41</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>11.77</td>
<td>1.27</td>
<td>42.60</td>
</tr>
<tr>
<td>Proline</td>
<td>4.32</td>
<td>0.62</td>
<td>20.35</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.93</td>
<td>0.74</td>
<td>25.04</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.82</td>
<td>0.95</td>
<td>31.93</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>3.72</td>
<td>0.51</td>
<td>17.24</td>
</tr>
<tr>
<td>Valine</td>
<td>5.67</td>
<td>0.79</td>
<td>26.52</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.95</td>
<td>0.23</td>
<td>7.31</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.00</td>
<td>0.45</td>
<td>15.10</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.83</td>
<td>1.22</td>
<td>40.96</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.15</td>
<td>0.35</td>
<td>11.90</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.62</td>
<td>0.54</td>
<td>17.98</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.34</td>
<td>0.68</td>
<td>11.22</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.40</td>
<td>0.33</td>
<td>10.99</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.14</td>
<td></td>
<td>1.01</td>
</tr>
<tr>
<td>Total</td>
<td>99.18</td>
<td>15.95</td>
<td></td>
</tr>
<tr>
<td>Recovery %</td>
<td>100.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calculated on the basis of a molecular weight of 47,200.

Fig. 3. Activity plotted against enzyme concentration of the dipeptidase. The reactions were followed by observing the fall in absorption at 275 μ of a solution of 0.08 μ dehydropeptide to which had been added 0.1 ml of pure dipeptidase to give the appropriate concentration of enzyme. All reactions were carried out at 35° in pH 8.1, 0.002 M Tris(hydroxymethyl)methylamino propane sulfonic acid buffer. The concentration of the enzyme in the reaction mixture was 0.893 μg per ml. The temperature throughout the reaction was 35°.

The irregular alterations in slope which produce deviations from symmetrical bell-shaped curves indicate the complexity of the effect of pH upon the peptidase-catalyzed reaction. However, there is evidently a

μmoles per min per mg and Kₘ 1.35 ± 0.04 m. The effect of pH upon rate was shown to be completely reversible at all pH values employed, and the rates measured over the pH range (7.2 to 7.4) where both buffers were used exhibited no buffer effect. The pH optimum of 7.6 was shown to be independent of substrate concentration over the range 8.00 × 10⁻⁸ M to 1.50 × 10⁻⁸ M glycyldehydrophenylalanine. Lineweaver-Burk (10) plots employed in the calculation of V_max and K_m values at various pH values are presented in Fig. 5.

The variation of maximal initial velocities and Michaelis constants with pH is shown in Fig. 6. The irregular alterations in slope which produce deviations from symmetrical bell-shaped curves indicate the complexity of the effect of pH upon the peptidase-catalyzed reaction. However, there is evidently a
general reciprocal relationship between values for \( K_m \) and \( V_{\text{max}} \); that is, when \( V_{\text{max}} \) increases \( K_m \) decreases, and the maximum value for \( V_{\text{max}} \) is within 0.1 pH unit of the minimum value for \( K_m \). A similar relationship between \( V_{\text{max}} \) and \( K_m \) is observed in the pH curves reported by Frieden and Alberty for the fumarase-catalyzed conversion of fumarate to l-malate (11). If the Michaelis constant can be assumed to approximate the dissociation constant for the enzyme-substrate complex, then these curves would suggest that an increase in affinity of enzyme for substrate results in an increased rate of decomposition of the complex formed. Such a direct relationship has been demonstrated in the case of copper-activated hydrolysis of glycyldehydrophenylalanine, where an increased association constant between the metal ion and peptide led to an increased rate in the rate of metal-activated hydrolysis (3). Clearly the contribution of \( K_n \), the enzyme-substrate dissociation constant, to \( K_m \), a kinetic constant operationally defined as the substrate concentration at which half the maximum velocity is reached, will have to be established before further analogy between the peptidase system and the model system can be drawn.

The method of Dixon (12, 13) has frequently been found useful in studies where the effect of pH on the kinetic behavior of an enzyme system is complex. According to the theoretical treatment of Dixon a plot of \( \log V_{\text{max}} \) against pH will give a curve made up of segments that change in slope in the pH range where dissociations of protons from the enzyme-substrate complex take place. The \( pK \) values of these dissociations are obtained by constructing linear segments with integral slopes (zero-, one-, or two-unit, positive or negative) which intersect at a point 0.3 units vertical distance from the experimentally determined graph. Such an analysis has been applied to the lower curve presented in Fig. 7. The points shown are experimentally determined. A reasonable agreement between experimentally measured points and the linear segments is found in the region of \( pK_{ES} \). However in the region of \( pK_{ES} \), the line drawn through the experimental points does not approximate a slope of +1. The deviation in this region will be a subject of future research. The \( pK \) values obtained from intersection points of the linear segments were employed to calculate a theoretical curve from the following equation.

\[
\log V_{\text{max}} = \log k(E) + p_{ES}
\]

where \( \log k(E) \) is a constant independent of pH calculated from values of \( V_{\text{max}} \) at several hydrogen ion concentrations, and

\[
p_{ES} = \log \left( 1 + \frac{(H^+) + K_{ES}}{K_{ES} (H^+)} \right)
\]

The theoretical curve is shown as the solid line in the lower part of Fig. 7. A good fit between the theoretical curve and the experimental points is demonstrated in pH region 7 to 9 but not in the region of the solid dissociation, \( pK_{ES} \).

Application of the Dixon method to a plot of \( pK_m \) against pH is shown in the upper curve of Fig. 7. In this case changes in slope reflect dissociations that occur in the substrate, the free enzyme, and the enzyme-substrate complex during the catalytic process. Where the curve appears concave upward the dissociation occurs in the enzyme-substrate complex. Where the curve appears concave downward the dissociation occurs in the substrate or free enzyme. Linear segments with unit slopes were constructed as described above, and the \( pK \) values were estimated from the points of intersection of these lines. The \( pK \) values were then employed to calculate a theoretical curve from the following equation.

\[
p\mu = pK + \log j_{ES} - \log j_E - \log j_S
\]

The \( pH \)-dependent functions \( f \) are given by

\[
f = 1 + \frac{(H^+) + K_3}{K_1 (H^+)}
\]

where the dissociation constants, \( K \), refer to \( K_{ES}, K_{ES}, \) or \( K_3 \), depending on which species the \( pH \) function describes. The value of \( pK \) is independent of \( pH \) and was calculated as a constant from data at several different \( pH \) values. Although the dissociation at \( pH 7.9 \) cannot be unequivocally assigned to the amino group of the substrate, it seems likely that this is the case since the dissociation constant of this group in glycyldehydro-
phenylalanine has been independently determined to be 7.8 by potentiometric titration (2). It has also been shown that the presence of this group is required for peptidase activity (1), and alteration of the substrate site by methylation results in a decreased rate of enzymic hydrolysis as well as a decreased rate of copper-activated hydrolysis (2). In addition it has been reported that the NH₂-terminal amino group of the peptide must be deprotonated prior to complexation with copper ion during copper-activated hydrolysis (3). Therefore it is suggested that this substrate site dissociates its proton during catalysis in order to provide a binding site for zinc in the peptidase-catalyzed system.

The solid line in the upper part of Fig. 7 represents the theoretical curve calculated from the estimated pK values and the experimentally measured pH values. Again, a good fit is observed in the pH range 7.6 to 9, but some deviation is observed on the acid side of the pH optimum. The pK values are assigned to free enzyme or enzyme-substrate according to the direction of concavity.

The pK values of the groups involved in the catalytic event obtained by a study of the effect of pH upon kinetic parameters can only be regarded as a first approximation. The tentative identification of these groups from the estimated pK values requires confirmation by additional independent data. Accordingly, work has been initiated with selective reagents to further identify the sites of the peptidase required for enzymatic hydrolysis of glycyldedehydrophenylalanine. Of particular interest is the group in the enzyme-substrate complex which dissociates a proton at pK 8.5. In this pH range the theoretical curves pass through the experimental points in both the V₅₀-pH plot and the pK₉-pH plot. There are few amino acid side chains with polar groups that dissociate near pH 8.5, but the dissociation of the sulfhydryl group of the cysteine residue has been reported to have a pK in the range 8.3 to 8.6 (14).

Preliminary results in our laboratory suggest that treatment of renal dipeptidase with either p-hydroxymercuribenzoate, α-iodoacetamide, or N-ethylmaleimide does not produce an alteration in enzyme activity. Conclusive evidence regarding the role of sulfhydryl groups in the catalytic action of the peptidase must await analysis of the sulfhydryl content of the enzyme and chemical analysis of hydrolysates of the enzyme following treatment with thiol reagents. The α-amino groups that occur at the NH₂-terminus of polypeptide chains exhibit a dissociation near pH 8 (15), and we are at present attempting to selectively modify these groups in renal dipeptidase.

The possibility exists that the dissociation at 8.5 results from the loss of a proton from a water molecule coordinated to zinc in the enzyme-substrate complex. In the model system previously described (3) copper-activated hydrolysis of glycyldedehydrophenylalanine occurs only after a proton has been dissociated from the hydration sphere of the metal-peptide complex. The pK of this dissociation has been estimated at 9.6 in the model system, and the pK for the dissociation of the 6-aquo-complex of zinc, Zn(H₂O)₆⁺⁺ → Zn(H₂O)₆OH⁺ + H⁺ has been reported to be 9.2 (16). These dissociations were measured in simple aqueous systems, and it is not unreasonable to suggest that a zinc complex involving coordination to protein sites as well as peptide groups would exhibit a lower pK. It is interesting to note that alkaline phosphatase and carbonic anhydrase, both zinc metalloenzymes that catalyze hydrolytic reactions, have been reported to exhibit dissociations near pK 8 during the catalytic process (17, 18). The suggestion that a water molecule coordinated to zinc in zinc metalloenzymes is involved in the catalytic process is an attractive possibility and worthy of future investigations.

REFERENCES

Amino Acid Composition and Effect of pH on the Kinetic Parameters of Renal Dipeptidase
Alex M. René and Benedict J. Campbell

J. Biol. Chem. 1969, 244:1445-1450.

Access the most updated version of this article at http://www.jbc.org/content/244/6/1445

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/244/6/1445.full.html#ref-list-1