Properties of a Specific Protease for Pyridoxal Enzymes and Its Biological Role

EIKI KOMINAMI, KEIKO KOBAYASHI, SACHIKO KOMINAMI, AND NOBUHIKO KATUNUMA
From the Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima, Japan

SUMMARY
A new protease which specifically acts on the apo-form of pyridoxal enzymes was discovered in rat small intestine and skeletal muscle. This enzyme was purified about 500-fold from the small intestine of vitamin B₆-deficient rats. Some properties of the enzyme and its action were studied. The enzyme split the apo-protein of ornithine transaminase into two products, a homogeneous smaller protein and an oligopeptide, and thereby inactivated the transaminase. The substrate enzyme, ornithine transaminase, and the larger product have \( k_{\text{cat}} \) values of 9.9 and 5.1, respectively. The enzyme inactivated all of the apo-pyridoxal enzymes tested, including serine dehydratase, ornithine transaminase, tyrosine transaminase and aspartate transaminase, but did not affect non-pyridoxal enzymes (glutamic dehydrogenase, lactic dehydrogenase, urease, and glutaminase), bovine serum albumin or some synthetic substrates. Addition of pyridoxal phosphate or a high concentration of pyridoxal had a protective effect against the specific protease, but another active coenzyme, pyridoxamine phosphate, did not.

The activity of this protease in the small intestine increased during vitamin B₆ deficiency. A reciprocal relation was found between the activity of this protease and that of ornithine transaminase in the small intestine.

Considerable evidence has accumulated in recent years indicating that synthesis and catabolism are both equally important in the regulation of the intracellular concentrations of enzymes (1, 2). There is now much information available on protein synthesis, but little is known of the mechanism and control of protein degradation. However, there have been several studies on protein catabolism in which the degradation of marker enzymes or proteins was measured in whole animals, isolated organs, slices, homogenates, and subcellular fractions. Grossman and Mavrides (3), Kenney (4), and Schimke (5) observed that inhibitors of protein synthesis prevented enzyme inactivation in whole animals. Levitan and Webb (6) also observed this in isolated perfused liver. These observations suggest that protein synthesis may be required for protein degradation. The requirement of energy for this process was demonstrated by Hershko and Tomkins (7) in cell cultures and by Bromstom and Jeffay (8) in homogenates. In these systems the processes involved were probably nonspecific intracellular degradations, but their nature was not clarified.

Coffey and de Duve (9) suggested that enzyme levels might be controlled by lysosomal proteases, but it seems unlikely that these would cause specific degradation of individual enzymes. Another mechanism of protein catabolism was shown by the work of Bonsignore et al. (10) who demonstrated a specific enzyme for the inactivation of glucose 6-phosphate dehydrogenase. However, this mechanism does not seem likely to explain the continual replacement of all proteins.

During studies on the mechanism of degradation of pyridoxal enzymes in vitamin B₆ deficiency, we found a new enzyme which may be important in the regulation of the intracellular concentration of pyridoxal enzymes in rat small intestine and skeletal muscle. Rapid and marked decrease of pyridoxal enzymes was observed in the small intestine of rats in vitamin B₆ deficiency (11). To investigate this mechanism further, we began our work in vitro by looking for the material which affects the decrease of the activity of ornithine transaminase. Purified ornithine transaminase was incubated with tissue homogenates prepared from several organs of vitamin B₆-depleted rats, and the change of ornithine transaminase activity was followed. The results showed that homogenates of small intestine and skeletal muscle contained some material which inactivated ornithine transaminase. Furthermore, we demonstrated that this material had the nature of an enzyme and that it attacked not only ornithine transaminase but also other pyridoxal enzymes. This new enzyme split the apo-protein molecule into a smaller protein and an oligopeptide under alkaline conditions.

This paper reports the purification and some properties of this enzyme, and the function of the enzyme in the regulation of the degradation of pyridoxal enzymes. A preliminary report of this work has appeared (12).

EXPERIMENTAL PROCEDURE

Materials—Pyridoxal phosphate was obtained from Chugai Pharmaceutical Co. Ltd., Tokyo, Japan. Other vitamin B₆ derivatives were obtained from Sigma and flavin adenine dinucleotide was from Boehringer-Mannheim. The enzymes
enzymes were converted to the apo-forms before use as sub-

and homoserine deaminase were obtained by the methods of

talline glutamic dehydrogenase from bovine liver and lactic
dehydrogenase from pig heart muscle were obtained commer-
cial and were assayed by measuring the rate of decrease in

and the percentage of inactivation was calculated. One unit
enzyme preparation. The mixture was incubated at 37°C and

Purification of pyridoxal enzymes specific splitting enzyme from

Acetone powder extract... 1,100 13,000 4,000,000 310 100
Ammonium sul-

Purification of pyridoxal enzymes specific splitting enzyme from

Acetone.. 1,000 6,100 4,100,000 660 100

Acetone... 200 4,220 5,040,000 1,190 123

| Fraction                  | Volume | Total protein | Total activity | Specific activity | Recovery % |
|---------------------------|--------|---------------|----------------|-------------------|------------|
| Acetone powder extract    | 1,100  | 13,000        | 4,000,000      | 310               | 100        |
| Ammonium sulfate          | 1,000  | 6,100         | 4,100,000      | 660               | 100        |
| Acetone                   | 200    | 4,220         | 5,040,000      | 1,190             | 123        |
| Acetone                   | 41     | 1,730         | 4,080,000      | 2,360             | 100        |
| DEAE-cellulose            | 2.4    | 48            | 1,240,000      | 25,800            | 30         |
| Sephadex G-100            | 1.0    | 4.8           | 620,000        | 130,000           | 15         |
per g of small intestine), and then the suspension was ground in a large mortar until the preparations were free from large lumps. The mixture was poured onto a Buchner funnel, and the residue was washed twice on the funnel with cold acetone. The material was sucked dry and the powder was crumbled and allowed to dry in the air at 4°C for a few hours. The dried powder was stored at -20°C. About 50 g of acetone powder were obtained from 450 g of small intestine. The acetone powder was mixed with 1,000 ml of 0.05 M potassium phosphate buffer, pH 7.5, and the mixture was stirred at 0°C for 20 min. It was then centrifuged at 10,000 × g for 10 min. At this step, increase of specific activity to 5- to 10-fold was observed.

Step 2—Ammonium sulfate fraction. Solid ammonium sulfate, 176 g per liter was added to the supernatant and the mixture was stirred for 30 min. The mixture was centrifuged at 10,000 × g for 10 min, and the precipitate was discarded. Then further ammonium sulfate was added to give 70% saturation (472 g per liter). The mixture was stirred and centrifuged and the precipitate was dissolved in 0.05 M potassium phosphate buffer, pH 7.5 (Buffer A) and dialyzed overnight against this buffer.

Step 3—First acetone fractionation. Ice-cold acetone (70 ml per 100 ml) was then added to the dialyzed suspension with stirring over a period of 5 min and the mixture was immediately centrifuged at 10,000 × g for 5 min, and the resulting precipitate was collected. This precipitate was dissolved in Buffer A and centrifuged briefly to remove insoluble material.

Step 4—Second acetone fractionation. The ice-cold acetone (40%, v/v) was added to the supernatant with stirring over a period of 5 min, and the mixture was immediately centrifuged at 10,000 × g for 5 min. To the supernatant solution additional cold acetone was added to give a final concentration of 60% (v/v), and the resulting precipitate was collected by centrifugation. This precipitate was dissolved in a minimum volume of Buffer A and centrifuged briefly to remove the insoluble material. The supernatant was passed through a Sephadex G-25 (5.5 × 20 cm) column equilibrated with Buffer A.

Step 5—DEAE-cellulose chromatography. The enzyme solution was applied to a DEAE-cellulose column (2.5 × 20 cm) equilibrated with Buffer A and the column was washed with 500 ml of the same buffer at a flow rate of 60 ml per hour. The column was then eluted step-wise with 300 ml of 0.05 to 0.20 M potassium phosphate buffer, pH 7.5, at a flow rate of 40 ml per hour; fractions of 6 ml were collected. The splitting enzyme was eluted in 0.20 M buffer. Fractions with high specific activity were pooled and solid ammonium sulfate (472 g per liter) was added to 70% saturation. After stirring for 30 min, the precipitate was collected by centrifugation at 10,000 × g for 10 min and dissolved in a small amount of Buffer A.

Step 6—Gel filtration on Sephadex G-100. The enzyme solution was applied in a Sephadex G-100 column (2.5 × 90 cm) equilibrated with Buffer A (Fig. 1a). The enzyme was excluded from the gel and was collected and concentrated to a final volume of about 1 ml in a collodion bag. Finally, the preparation was columns were equilibrated with 0.1 M potassium phosphate buffer pH 7.5, at a flow rate of 15 ml per hour. The purified splitting enzyme was applied to columns in combinations with blue dextran (mol wt 2,000,000) and one of following markers: sperm whale myoglobin (mol wt 17,900), beef pancreatic chymotrypsinogen A (mol wt 25,000), ovalbumin (mol wt 45,000), and bovine serum albumin (mol wt 67,000). The elution patterns were measured by determination of protein, and that of the splitting enzyme (G.S.S. Enzyme) was determined by assay of activity.

FIG. 1. a, Sephadex G-100 chromatography. The enzyme was eluted from the column with 0.05 M potassium phosphate buffer at a flow rate of 55 ml per hour. Fractions of 6 ml were collected and assayed for the absorbance at 280 nm and the splitting enzyme activity. b, polyacrylamide gel electrophoresis of the purified pyridoxal enzymes specific splitting enzyme. Fifty micrograms of enzyme were applied and migration was from top to bottom of the gel. Each gel was 6 cm long. c, determination of the molecular weight of the pyridoxal enzymes specific splitting enzyme by gel filtration on columns of Sephadex G-100 (2.0 × 80 cm). The

Downloaded from http://www.jbc.org/ by guest on March 24, 2020
subjected to disc gel electrophoresis at 4°C with a constant current of 2 mA per tube in 0.05 M Tris-0.38 M glycine buffer. The preparation gave one major protein band and one minor protein band which stained with Amido black (Fig. 1b). Elution of slices of an unstained control gel permitted demonstration of the splitting enzyme activity coincident with the major protein band. The specific splitting enzyme. Enzyme activity was assayed under standard conditions except that the concentration of the substrate, ornithine transaminase, was varied. The inset shows a Lineweaver-Burk plot of data from this figure.

Some Properties of Purified Enzyme

The molecular weight of the purified splitting enzyme was estimated by gel filtration on a Sephadex G-100 column as 31,000 using the method of Andrews (35), as shown in Fig. 1c.

The purified preparation of the splitting enzyme showed high specificity for pyridoxal enzymes as shown in Table II. Even with prolonged incubation times the activity of non-pyridoxal enzymes tested (glutamic dehydrogenase, lactic dehydrogenase, urease, and phosphate-independent glutaminase) was not affected. The protease activity of the splitting enzyme was assayed with synthetic substrates. Larger amounts of enzyme were used in this experiment since, in general, synthetic substrates are less sensitive than protein substrates to proteases. No activity was observed with trypsin substrates (N-benzoyl-L-arginine ethyl ester, L-arginine methyl ester, p-toluene sulfonyl-L-arginine methyl ester, L-lysine methyl ester, p-toluene sulfonyl-L-lysine methyl ester) or chymotrypsin substrates (N-acetyl-L-tyrosine ethyl ester, L-tyrosine ethyl ester).

The optimum pH for inactivation of ornithine transaminase was studied with both crude and purified preparations and both pH curves had an optimum at pH 9.0 (Fig. 2).

The relationship between the substrate concentration and reaction velocity was studied, the results of which are shown in Fig. 3. The Michaelis constant of the splitting enzyme for the substrate, ornithine transaminase, was calculated as 1.52 \times 10^{-5} M, assuming that the molecular weight of ornithine transaminase is 132,000 as reported by Peraino et al. (30).

Effect of Vitamin B6 Derivatives on Activity of Splitting Enzyme

The apo-form of the enzymes was inactivated by the splitting enzyme. The effects of vitamin B6 derivatives and other vitamins on the inactivation of these enzymes were studied by incubating apo-ornithine transaminase with compounds before adding the splitting enzyme. Pyridoxal phosphate and pyridoxal protected the enzymes against the splitting enzyme.

![Fig. 2 (left). pH-activity curve of the pyridoxal enzyme specific splitting enzyme. Buffer was used at a final concentration of 50 mM potassium phosphate buffer, pH 6.0 to 8.0; Tris-HCl buffer, pH 8.0 to 9.0; glycine-sodium hydroxide buffer, above pH 9.0.](http://www.jbc.org/)

![Fig. 3 (center). Effect of concentration of substrate enzyme (ornithine transaminase) on the activity of pyridoxal enzymes specific splitting enzyme.](http://www.jbc.org/)

![Fig. 4 (right). Effect of concentration of pyridoxal phosphate and pyridoxal on the activity of the pyridoxal enzymes specific splitting enzyme.](http://www.jbc.org/)
Ba derivatives and other vitamins, 50 units of apo-ornithine transaminase was added to the eluted enzyme passing the reaction mixture through a column of Sephadex G-25. with 0.5 mM and 5 mM pyridoxal phosphate for 30 min, and then the protective effect of pyridoxal phosphate or 30 times more pyridoxal than pyridoxal phosphate is almost the same as the pyridoxamine phosphate (37). Thus, the addition of pyridoxal phosphate and pyridoxamine phosphate, did not have protective effects. It is interesting that pyridoxamine phosphate had no protective effect since functionally the pyridoxamine phosphate form of the enzyme is as active as the pyridoxal phosphate form in the transaminase reaction. The same result was recognized when the pyridoxamine phosphate form was made by the incubation of the pyridoxal phosphate form with ornithine in the absence of α-ketoglutarate.

The protection was most effective when apo-ornithine transaminase was incubated with pyridoxal phosphate and thus converted to the holo-form. Pyridoxal phosphate also protected tyrosine transaminase and serine dehydratase from inactivation. The concentrations of pyridoxal phosphate and pyridoxal required for 50% protection were calculated as 6.0 × 10⁻⁴ M and 1.0 × 10⁻⁴ M, respectively (Fig. 4). This concentration of pyridoxal phosphate is almost the same as the Kₘ value of ornithine transaminase for pyridoxal phosphate (37). Thus, the addition of pyridoxal phosphate or 30 times more pyridoxal than pyridoxal phosphate should be equally effective in protecting the enzyme against inactivation.

The effect of pyridoxal phosphate on a purified preparation of the splitting enzyme was studied by incubating the enzyme with 0.5 mM and 5 mM pyridoxal phosphate for 30 min, and then passing the reaction mixture through a column of Sephadex G-25. Then, apo-ornithine transaminase was added to the eluted enzyme solution, and the splitting enzyme activity was assayed. Pyridoxal phosphate was found to have no effect on the splitting enzyme, suggesting that the splitting reaction is not dependent on pyridoxal phosphate.

### Analysis of Reaction Products

To examine the products of the reaction, crystalline apo-ornithine transaminase was partially inactivated by incubation with purified splitting enzyme and then the reaction mixture was immediately applied to a Sephadex G-100 column. The elution profile is shown in Fig. 5a. The first solid line represents intact ornithine transaminase and the second solid line is the protein split from ornithine transaminase by the enzyme. The second peak did not have ornithine transaminase activity. The splitting enzyme gave the third peak indicated by a solid line. The fourth peak is due to a second product derived from ornithine transaminase, which seems to be an oligopeptide. The elution pattern shown in Fig. 5a was obtained with holo-ornithine transaminase as substrate. All of the ornithine transaminase activity added was recovered in the first peak, and no other protein or peptide liberated from substrate. Ornithine transaminase was detected on the chromatogram. These results indicate that the enzyme splits ornithine transaminase into two products, a smaller protein and an oligopeptide. Therefore, the splitting enzyme seems to be a specific endopeptidase. To examine these products further, the first peak, apo-ornithine transaminase, and the second peak eluted from the Sephadex G-100 column were subjected to ultracentrifugal analysis (Fig. 6).

Both proteins appeared to be homogeneous on ultracentrifugation and in the standard buffer had χ² values of 9.9 and 5.1, respectively. On electrophoresis on a cellulose acetate membrane they also both appeared homogeneous and had similar mobilities. The second peak had no ornithine transaminase activity but could not be distinguished from the latter antigenically. On analysis on Ouchterlony double diffusion plates, the antibody specific for native ornithine transaminase gave a single precipitation line with the material in the second peak.

### Further Degradation of Ornithine Transaminase by Trypsin

It was of interest to study whether degradation of pyridoxal enzymes by the splitting enzyme could be followed by the action of intracellular nonspecific proteases. Apo-ornithine transaminase was first inactivated by the splitting enzyme and then the larger product was isolated by chromatography on Sephadex G-100. This fraction was then incubated with trypsin in 0.1 M potassium phosphate buffer, pH 7.5. Simultaneously, native apo-ornithine transaminase was subjected to trypsin.

As shown in Fig. 7, apo-ornithine transaminase and bovine serum albumin were scarcely degraded by trypsin, while the product was markedly degraded.
Fig. 6. Sedimentation patterns of apo-ornithine transaminase and its product released by the splitting enzyme specific for pyridoxal enzymes. The product was obtained by applying the reaction products of apo-ornithine transaminase released by the splitting enzyme to a Sephadex G-100 column, as shown in Fig. 5. Both peaks on the chromatogram, apo-ornithine transaminase (10.8 mg per ml) in 0.1 M potassium phosphate buffer, pH 7.5, and the product (11.5 mg per ml) in the same buffer (b) were centrifuged independently in a Hitachi analytical centrifuge, type 1, at 5°. Sedimentation is from left to right. Pictures were taken at 0-min intervals after attaining the maximum speed of 60,000 rpm.

**Tissue Distribution of Enzyme**

The distribution of the splitting enzyme was studied using crude preparations of tissues from vitamin B₆-deficient rats. Among the tissues examined, the small intestine had high activity and skeletal muscle low activity. Extremely low activity was observed in other organs (liver, kidney, heart, spleen, brain, stomach, large intestine) compared with that of two organs described previously. However, it is possible that the enzyme may be present in a latent form in other organs.

**Effect of Dietary Conditions on Enzyme Activity**

The activity of the splitting enzyme in the small intestine of animals under various dietary conditions was investigated. Activity increased in vitamin B₆ deficiency, as shown in Table IV, at a time when ornithine transaminase activity in the small intestine, determined as an indicator of B₆ deficiency, was greatly reduced. The activity of the splitting enzyme did not increase in vitamin B₆ or niacin deficiency but was increased in rats fed on high protein diets.

**Relation between Activity of Enzyme and of Ornithine Transaminase in Small Intestine**

In order to get information on the roles of the splitting enzyme in vivo, activity of both the splitting enzyme and of ornithine transaminase was assayed in crude extracts of small intestine of rats fed on vitamin B₆-deficient diet for various periods. As shown in Fig. 8, a reciprocal relation between both activities was observed. A double logarithmic plot of both activities yields a straight line with the range of ornithine transaminase activity from 1.0 to 0.2 unit per mg of protein. Linearity of the double logarithmic plots permits application of the following equations to express the relation between both activities.

\[
\log [\text{Ornithine transaminase}] = \log 2.2 - 0.5 \log [\text{Splitting enzyme}]
\]
not act as coenzyme of pyridoxal enzymes. Peraino et al. (36) found that 1 molecule of ornithine transaminase contains about 60 lysine residues and 2 special lysine residues among them are sites to bind with pyridoxal phosphate. It is expected that addition of 30 times more pyridoxal than pyridoxal phosphate should form a Schiff base with all of the lysine residues including these two special lysine residues in the ornithine transaminase molecule.

On the other hand, the pyridoxamine phosphate form of pyridoxal enzymes, as well as the apo-form, is susceptible to the splitting enzyme (Table III). Therefore, these two observations suggest that special lysine residues, which only form a Schiff base with pyridoxal phosphate, must remain free when pyridoxal enzymes are available as substrate for the splitting enzyme. The latter observation also suggests that transaminases may be susceptible to degradation by the splitting enzyme at some time during resonance, since the pyridoxamine phosphate form is as active as the pyridoxal phosphate form in the transaminase reaction.

Fig. 8. A reciprocal relation between the splitting enzyme specific for pyridoxal enzymes activity and ornithine transaminase activity in small intestine during the development of vitamin B$_6$ deficiency. Rats weighing 100 g were maintained on vitamin B$_6$-deficient diets for the various periods before experiments. Crude preparations from small intestine were obtained as described under "Experimental Procedure," and both activities of the splitting enzyme and of ornithine transaminase were assayed. The inset shows a double logarithmic plot of data.

\[
\text{[Ornithine transaminase]} = \frac{2.3}{\sqrt{[\text{Splitting enzyme}]}}
\]

This equation suggests the possibility that concentration of ornithine transaminase as a representative of pyridoxal enzymes is controlled by the splitting enzyme. In considering this problem, the intracellular compartmentalization of the splitting enzyme and that of pyridoxal enzymes must be taken into consideration.

**DISCUSSION**

We named the new enzyme described in this paper a group-specific splitting enzyme for pyridoxal enzymes on the basis of the following four experimental observations.

1. The enzyme showed strict group specificity for pyridoxal-dependent apo-enzymes and did not affect any other non-pyridoxal enzymes or proteins or synthetic substrates tested (Table II).

2. Addition of pyridoxal phosphate completely protected apo-pyridoxal enzymes from inactivation by this enzyme (Table III).

3. The activity of this enzyme increased in vitamin B$_6$ deficiency, but not in vitamin B$_3$ or nicotinamide deficiency (Table IV). The exact reason for this is unknown but it indicates that there must be some relation between changes in the activity of this enzyme and the intracellular concentration of vitamin B$_6$.

This enzyme inactivated apo-ornithine transaminase, \( \varepsilon_{20,\text{w}} = 9.9 \) by splitting it into an oligopeptide and a smaller protein \( \varepsilon_{20,\text{w}} = 5.1 \).

The enzyme has an alkaline pH optimum. In this property it differs from lysosomal proteases which usually have an acidic pH optimum.

As indicated in Table III, a large amount of pyridoxal had a protective effect against the splitting enzyme, although it did not act as coenzyme of pyridoxal enzymes. Peraino et al. (36) and Matsuzawa et al. (17) found that 1 molecule of ornithine transaminase contains about 60 lysine residues and 2 special
Acknowledgment—We wish to thank Dr. Mutsumi Muramatsu for his gift of synthetic substrates for proteases and helpful advice.

REFERENCES

1. Schimke, R. T., Swenney, F. W., and Berlin, C. M. (1965) J. Biol. Chem. 240, 322-331
2. Segal, H. L., and Kim, Y. S. (1965) J. Cell. Comp. Physiol. 65, Suppl. 1, 11
3. Grossman, A., and Maurides, C. (1967) J. Biol. Chem. 242, 1389-1405
4. Kenney, F. T. (1967) Science 155, 525
5. Schimke, R. T. (1967) Nat. Cancer Inst. Monograph 27, 301
6. Levitan, I. B., and Webb, T. E. (1969) J. Biol. Chem. 244, 4094-4098
7. Hersko, A., and Tomkins, G. M. (1971) J. Biol. Chem. 246, 710-714
8. Brostrom, C. O., and Jeffay, H. (1970) J. Biol. Chem. 245, 4001-4008
9. Coffey, J. W., and de Duve, C. (1968) J. Biol. Chem. 243, 3255-3263
10. Bonsignore, A., de Flora, A., Mangiarotti, M. A., Lorboni, I., and Alemi, S. (1968) Biochem. J. 106, 147
11. Katunuma, N. (1971) Protein Nucl. Acid Enzyme 18, 299
12. Katunuma, N., Komiss, E., and Kominami, S. (1971) Biochem. Biophys. Res. Commun. 45, 70
13. Woolley, D. W. (1950) Ann. N. Y. Acad. Sci. 52, 1335
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
15. Matsuzawa, T., Katunuma, N., and Matsumura, N. (1968) Biochem. Biophys. Res. Commun. 32, 161
16. Valeriote, F. A., Authier, F., Tomkies, G. M., and Riley, D. (1969) J. Biol. Chem. 244, 3618-3624
17. Nakagawa, H., Kimura, H., and Miura, S. (1967) Biochem. Biophys. Res. Commun. 23, 359
18. Huzino, A., Konishi, M., Yoshida, T., and Katunuma, N. (1962) Vitamins (Japan) 27, 148
19. Matsumo, Y., and Greenberg, D. M. (1958) J. Biol. Chem. 230, 601-611
20. Nishii, Y., Shimizu, T., and Ando, A. (1969) Vitamins (Japan) 40, 442
21. Matsuo, Y., and Greenberg, D. M. (1959) J. Biol. Chem. 234, 507-515
22. Katunuma, N., Okada, M., Matsuzawa, T., and Otsuka, Y. (1965) J. Biochem. 47, 446
23. Rosen, E., Harding, H. R., Milmolland, R. J., and Nichol, C. A. (1963) J. Biol. Chem. 238, 3725-3729
24. Sayer, F. W., and Greenberg, D. M. (1956) J. Biol. Chem. 220, 787-797
25. Kames, A. (1955) J. Clin. Invest. 34, 131
26. Bieles, W. J., and Feinman, G. M. (1966) Methods Enzymol. 9, 288
27. Olson, J. A., and Anfinson, C. B. (1965) J. Biol. Chem. 246, 67
28. Katunuma, N., Kuroda, Y., Sanada, Y., Towatari, T., Tomino, I., and Morris, H. P. (1970) in Advances in Enzyme Regulation (Weber, G., ed) Vol. 5, p. 281, Pergamon Press, New York
29. Katunuma, N., Huzino, A., and Tomino, I. (1967) in Advances in Enzyme Regulation (Weber, G., ed) Vol. 5, p. 55, Pergamon Press, New York
30. Ruiz-Herra, J., and Gonzalez, J. (1969) Anal. Biochem. 36, 366
31. Roberts, P. S. (1966) J. Biol. Chem. 232, 265-291
32. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404
33. Schachman, H. K. (1967) Methods Enzymol. 4, 52
34. Clausen, J. (1969) in Laboratory Techniques in Biochemistry and Molecular Biology (Weber, T. S., and Work, E., eds) Vol. I, Part 3, p. 397, North-Holland Publishing Co., Amsterdam and London
35. Andrews, P. (1964) Biochem. J. 91, 222
36. Peraino, C., Bunville, L. G., and Talmisian, T. N. (1969) J. Biol. Chem. 244, 2241-2249
37. Katunuma, N., Matsuda, T., and Tomino, I. (1964) J. Biol. Chem. 239, 499
Properties of a Specific Protease for Pyridoxal Enzymes and Its Biological Role
Eiki Kominami, Keiko Kobayashi, Sachiko Kominami and Nobuhiko Katunuma

J. Biol. Chem. 1972, 247:6848-6855.

Access the most updated version of this article at http://www.jbc.org/content/247/21/6848

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/247/21/6848.full.html#ref-list-1