MODES OF PROTEOLYSIS OF CYSTATHIONASE AND ORNITHINE AMINOTRANSFERASE BY SERINE PROTEASE FROM THE RAT SMALL INTESTINE

Yoshiko BANNO, Eiki KOMINAMI, and Nobuhiko KATUNUMA¹

*Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine,
Tokushima University, Tokushima 770, Japan*

(Received April 28, 1978)

Summary The mechanisms of proteolysis of two apo-forms of pyridoxal enzymes, cystathionase [EC 4.2.1.15] and ornithine aminotransferase [EC 2.6.1.13] by serine protease from the rat small intestine were compared.

The apo-forms of these two pyridoxal enzymes are susceptible to the serine protease, whereas the holo-forms of the enzymes are not. Pyridoxal phosphate, the coenzyme of these two enzymes, prevented their inactivation by the serine protease. The difference in susceptibility of the apo- and holo-forms to the serine protease was due to the difference in their conformations.

The time course of inactivation of cystathionase by the protease was apparently biphasic. During the first phase of inactivation, disappearance of the band corresponding to the native protomer, with a molecular weight of 47,000, was accompanied by accumulation of new material with a molecular weight of 39,000. In the late stage of proteolysis, extensive degradation of the large molecular weight intermediate was observed. This large intermediate product was isolated and found to compete with intact cystathionase as a substrate for the protease. Proteolysis of cystathionase was accompanied by both dissociation of the enzyme molecule and loss of its antigenicity.

Limited proteolysis of ornithine aminotransferase apoenzyme by the serine protease resulted in formation of a large molecular weight product similar to the native apoenzyme, but with nicks in the molecule. Dodecylsulfate-polyacrylamide gel electrophoresis showed that the apoenzyme is degraded to intermediate forms (molecular weight 41,500 and 15,000) and later, to stable forms (molecular weight 25,500 and 13,500).

¹ 坂野喜子, 木南英紀, 勝沼信彦

Abbreviation: SDS, sodium dodecylsulfate.

Previously, we reported the existence of several serine proteases in various organs of rats, and suggested that they might be involved in intracellular protein degradation (1-4). These proteases were found to inactivate the apo-forms of some pyridoxal enzymes and the apo-form of ornithine aminotransferase was found to be inactivated by limited proteolysis with formation of a large molecular weight intermediate product (3). Therefore, we investigated whether other apo-pyridoxal enzymes were also degraded by limited proteolysis by the serine protease.

This paper reports studies on the limited proteolysis of cystathionase apoenzyme and ornithine aminotransferase by the serine protease from the rat small intestine.

MATERIALS AND METHODS

Materials. Phenylmethyl-sulfonyl fluoride was obtained from Sigma Chemical Co. The markers used for determination of molecular weights, chymotrypsinogen, myoglobin and cytochrome *c*, were products of Mann Research Laboratories. Glyceraldehyde-3-phosphate dehydrogenase and glutamate dehydrogenase (beef liver) were purchased from Boehringer, Mannheim. γ -Globulin (HG II), aldolase (rabbit muscle) and soybean trypsin inhibitor were from Sigma Chemical Co. Egg albumin (chicken) was obtained from Seikagaku Kogyo Co. Bovin serum albumin was a product of Miles Laboratories. Acrylamide was from Eastman Kodak Co. Sephadex G-100 was from Pharmacia.

Enzyme preparation and assay. Ornithine aminotransferase was purified from rat liver to a homogeneous crystalline state and assayed as described previously (5). Ornithine aminotransferase apoenzyme II was prepared as described previously (6). Cystathionase was purified from rat liver as described by MATSUO and GREENBERG (7). The holoenzyme was resolved by the method of MUSHAHWAR and KOEPPE (8); that is, the holoenzyme (25 mg) was dialysed for one hour against 1 liter of 50 mM potassium phosphate buffer, pH 7.5, containing 1 mM hydroxylamine, 10 mM EDTA and 7.5 mM mercaptoethanol, and then the solution was applied to a column of Sephadex G-25, previously equilibrated with the same buffer without hydroxylamine. The eluted enzyme was almost completely in the apo-form. Cystathionase activity was determined with 50 mM homoserine as substrate by the method of SAYRE and GREENBERG (9).

Estimation of molecular weight. Molecular weights were determined by Sephadex G-100 column (2 $\times$ 90 cm) chromatography or polyacrylamide gel electrophoresis in the presence of 10% sodium dodecylsulfate (SDS). For chromatography, γ -globulin was used as a marker of the void volume and bovine serum albumin, soybean trypsin inhibitor, myoglobin and cytochrome *c* were used as markers of known molecular weight. The molecular weight of the apoenzyme of cystathionase was calculated as 160,000 from the results of CHURCHICH and DUPOURQUE (10).

Protein determination. Protein concentrations were determined by the method of LOWRY *et al.* (11) using crystalline bovine serum albumin as the standard.

Product analysis. The proteolytic cleavage of cystathionase apoenzyme was measured with the serine protease from the small intestine in 0.1 M potassium phosphate buffer, pH 8.0, containing 7 mM 2-mercaptoethanol. Reaction mixtures were incubated at 37°C and at intervals, 10- μ l samples were taken for assay of residual cystathionase activity. For electrophoretic analysis of the products, 50- μ l samples were mixed with an equal volume of boiling dodecylsulfate solution (2% dodecylsulfate, 2% mercaptoethanol and 20 mM phosphate buffer, pH 7.2) to stop the reaction. Then the mixtures were heated at 100°C for 3 min and applied to the gels. The course of proteolysis of cystathionase apoenzyme was followed using two systems: the "1/1 protease system," consisting of 1 ml of 0.5 mg of cystathionase apoenzyme and 0.45 mg of the protease, incubated at 37°C; and the "10/1 protease system," consisting of 1 ml of 0.5 mg of cystathionase apoenzyme and 0.045 mg of the protease, incubated at 0°C.

Polyacrylamide gel electrophoresis. Gels containing SDS were prepared as described by WEBER and OSBORN (12). Electrophoresis was carried out in 10% acrylamide gel. Immediately after electrophoresis, gels were stained for 2 hr with 0.1% Coomassie brilliant blue in 7.5% acetic acid and then destained with 7.5% acetic acid in 25% methanol.

Immunochemical studies. Antiserum against purified cystathionase holoenzyme from rat liver was prepared by the method of PASCAL *et al.* (13). Ouchterlony gel double diffusion analysis was performed as described by CLAUSEN *et al.* (14).

RESULTS

Conformation of the enzymes and effects of vitamin B₆ derivatives on their susceptibility to the serine protease

KHAIRALLAH and PITOT (15) and KATUNUMA (16) found that an increase in the activities of pyridoxal enzymes caused by pyridoxine administration was due to the ability of pyridoxal phosphate to protect these enzymes from degradation. Previous studies (1) showed that the apo-forms of cystathionase and ornithine aminotransferase were susceptible to the serine protease, whereas the holo-forms were not. To confirm these results we incubated the apo-forms of the two enzymes with vitamin B₆ derivatives and then measured their degradation by protease. As shown in Table 1, addition of excess pyridoxal phosphate or pyridoxamine phosphate, which is the coenzyme of ornithine aminotransferase, reduced the rate of their degradation, whereas pyridoxine phosphate and pyridoxamine had no effect. Moreover, pyridoxamine phosphate, which is not a coenzyme of cystathionase, did not protect this enzyme from inactivation by the serine protease, the opposite is true for pyridoxal phosphate. Vitamin B₆ derivatives were found to have no effect on the hydrolysis of N-acetyl-L-tyrosine ethyl ester by the serine protease.

Table 1. Effects of vitamin B₆ derivatives on susceptibility to serine protease and numbers of exposed tryptophan residues of holo- and apo-forms of cystathionase and ornithine aminotransferase. Reaction mixture containing the indicated amounts of vitamin B₆ derivatives, 18 units of cystathionase apoenzyme or 50 units ornithine aminotransferase apoenzyme and 200 mM potassium phosphate buffer, pH 8.0, were incubated for 30 min at 37°C. The reaction then was initiated by adding the serine protease. The difference of absorbance of the enzymes at 292 nm in two different solvents (0.05 M potassium phosphate buffer and 40% ethylene glycol) was measured as described in the previous paper (3). Values are means $\pm$ S.D. for eight experiments.

Addition	Cystathionase				Ornithine aminotransferase			
	Final concentration (M)	Rate of degradation (%)	Exposed tryptophan residues (number)		Final concentration (M)	Rate of degradation (%)	Exposed tryptophan residues (number)	
None		100	7.33 $\pm$ 0.25			100	4.29 $\pm$ 0.6	
Pyridoxal phosphate	1 $\times$ 10 ⁻⁵	17	5.81 $\pm$ 0.49		2.7 $\times$ 10 ⁻⁵	12	2.15 $\pm$ 0.4	
Pyridoxamine phosphate	1 $\times$ 10 ⁻⁴	115	—		2.3 $\times$ 10 ⁻⁴	6.6	—	
Pyridoxine phosphate	1 $\times$ 10 ⁻⁴	110	—		2.3 $\times$ 10 ⁻⁴	90	—	
Pyridoxamine	1 $\times$ 10 ⁻⁴	—	—		2.7 $\times$ 10 ⁻⁴	90	—	

SANADA *et al.* (6) observed that resolution of the coenzyme from ornithine aminotransferase is accompanied by dissociation of the enzyme with increase in the number of sulfhydryl groups. On the other hand, resolution of the coenzyme from cystathionase does not alter its molecular weight. However, the apo-forms of both enzymes are susceptible to the serine protease, whereas the holo-forms are not. The numbers of exposed tryptophan residues were studied by measuring the difference spectra of the apoenzymes and holoenzymes produced by various concentrations of ethylene glycol as described in a previous paper (3). From the results, it was calculated that 5.8 tryptophan residues are exposed in the holo-form, and 7.3 in the apo-form of cystathionase. As the total tryptophan content of cystathionase is 14 residues (17), 41 percent are exposed in the holo-form and 11 percent more in the apo-form (7.3 of the total 14).

Further, the resolution of the pyridoxal phosphate form of ornithine aminotransferase apparently caused an increased exposure of tryptophan residues.

Mode of proteolysis of cystathionase

Proteolysis of the apo-form of cystathionase by the serine protease from the small intestine in the "10/1 protease system" at 0°C was followed by measuring the decrease in enzyme activity and change in the molecular weight of the product separated by SDS-polyacrylamide gel electrophoresis. The results in Fig. 1 show that the loss of activity corresponded quantitatively to the loss of material migrating in the position of intact protomers. Moreover, with progressive loss of enzyme activity a more rapidly migrating band appeared and increased in intensity. The molecular weights of the intact protomer and the material in this protein band (product) were determined to be about 47,000 and about 39,000 by SDS-polyacrylamide gel electrophoresis (Fig. 2). These results were obtained by SDS-electrophoresis, which causes dissociation of protein, so it was not shown whether or not limited proteolysis of cystathionase was accompanied by dissociation of the enzyme molecule. For examination of this problem, the reaction mixture obtained by incubation of the apo-form of cystathionase with the protease (10:1) at 0°C for 7 hr was applied to a column of Sephadex G-100. Three peaks of material with absorption at 280 nm were eluted from the column, as shown in Fig. 3-(A). The first peak was that of the remaining cystathionase and the third peak was that of the protease, whereas the second peak showed neither activity. The elution volumes of these three peaks corresponded to apparent molecular weights of about 160,000, 35,000 and 25,000, respectively. When, as control, the apo-form of cystathionase was applied to the same column under the same conditions, only one peak (the first peak) was observed. The materials in these fractions were analyzed by SDS-acrylamide gel electrophoresis (Fig. 3-(B)). The material in the first peak coincided with native cystathionase subunits (molecular weight 47,000) and that in the third peak corresponded to the protease. The material in the second peak was a single protein with a molecular weight of 38,000, which appeared to be a large molecular weight intermediate product. These results show that on limited proteolysis,

Fig. 1. Kinetics of proteolysis of cystathionase by serine protease (10:1) at 0°C. The incubation conditions are described under METHODS. Samples were removed at the indicated times for assay of cystathionase activity and for analysis by SDS-polyacrylamide gel electrophoresis. At the times indicated, 5 μ l of 10 mM phenylmethylsulfonyl fluoride were added to 50 μ l of the enzyme-treated cystathionase and the remaining activity of cystathionase was measured. (●—●) Percentage residual activity of cystathionase with homoserine as substrate. (A): intact protomer, (B): product. Numbers (1) to (7) show products after reaction times of 0, 1, 2, 3, 4, 5 and 6 hr, respectively.

cystathionase yielded a single product with a molecular weight of 35,000–38,000. The product was not present in an associated form in the first peak eluted from the column, because this material formed a single band on SDS-acrylamide gel electrophoresis.

For examination of subsequent stages of degradation, proteolysis of the apo-form of cystathionase was followed using the "1/1 protease system." As shown in Fig. 4, the time course of enzyme inactivation by the protease is apparently biphasic, suggesting that two reactions are involved in the inactivation. During the first phase of inactivation, proteolysis was similar to that with the 10/1 protease system: that is, disappearance of the band corresponding to the native protomer was accompanied by accumulation of material in a new protein band.

It is noteworthy that no other band besides that of the large molecular weight

Fig. 2. Estimation of molecular weights of the native protomer of cystathionase and the large molecular weight intermediate by SDS-polyacrylamide gel electrophoresis. Polyacrylamide gels (10% containing SDS) were used as described under METHODS. The marker proteins were: (1) glutamate dehydrogenase; (2) egg albumin; (3) aldolase; (4) chymotrypsinogen; (5) myoglobin. Migration is shown relative to that of bromophenol blue. (A): cystathionase, (B): product, (C): protease.

Fig. 3. (A) Separation of reaction products by Sephadex G-100 column chromatography. Cystathionase was incubated with the serine protease (10:1) for 7 hr at 0°C. The reaction mixture was applied to a Sephadex G-100 column (2.0 × 90 cm) equilibrated with 0.1 M potassium phosphate buffer, pH 7.5, containing 5 mM mercaptoethanol and 10 mM EDTA. (○—○) Optical density of 280 nm; (●—●) cystathionase activity; (△—△) protease activity. The marker proteins were: (1) γ -globulin; (2) bovine serum albumin; (3) egg albumin; (4) soybean trypsin inhibitor; (5) myoglobin; (6) cytochrome *c*.

(B) Identification of protein fractions from Sephadex G-100 by SDS-polyacrylamide gel electrophoresis. The protein peaks obtained from Sephadex G-100 were concentrated in a collodion bag and analyzed on 10% polyacrylamide gels containing SDS. (A): First peak; (B): second peak; (C): third peak.

Fig. 4. Kinetics of proteolysis in the "1/1 protease system." The reaction mixture (1 ml) containing 0.5 mg of cystathionase apoenzyme, 0.4 mg of the serine protease, 7 mM mercaptoethanol and 10 mM EDTA in solution containing 0.1 M potassium phosphate buffer, pH 8.0, was incubated at 37°C. At the times indicated, 10- μ l samples were removed for assay of remaining cystathionase activity and 50- μ l samples for analysis on SDS-polyacrylamide gel.

intermediate appeared on SDS-acrylamide gel electrophoresis. Extensive degradation of the large molecular weight intermediate probably occurs at a late stage of proteolysis. A linear increase in ninhydrin positive material in the trichloroacetic acid soluble fraction was observed during the reaction, even when the rate of inactivation was slow, indicating that the large molecular weight intermediate was degraded to peptides of low molecular weight or to amino acids. The biphasic mode of inactivation can be explained by supposing that subsequent proteolytic steps are faster than the initial cleavage of the native enzyme and that the large intermediate product formed initially competes with the intact apo-cystathionase as substrate for the protease. This possibility was confirmed by measuring the initial rate of the reaction of the apo-form of cystathionase with the protease in the presence or absence of the large product.

The large product showed competitive inhibition with the native apo-form of cystathionase (Fig. 5): that is, addition of the large molecular weight intermediate (0.6 mg) reduced the rate of degradation of cystathionase apoenzyme (less than 0.61 mg). The K_m value for cystathionase protein and the K_i value for the large intermediate product were calculated to be 3.4×10^{-6} M and 1.6×10^{-5} M,

Fig. 5. Effect of the large molecular weight intermediate product on inactivation of cystathionase apoenzyme by serine protease. Proteolysis was performed at 0°C with 0.4 mg of serine protease in 1 ml of solution containing 0.1 M potassium phosphate buffer, pH 8.0, 7 mM mercaptoethanol and 10 mM EDTA in a final volume of 0.3 ml. After 2 hr of incubation of reaction mixtures containing various concentrations of cystathionase apoenzyme with or without 0.6 mg of the large molecular weight intermediate, the residual activities were measured. (▼-▼) With the intermediate; (●-●) without the intermediate.

respectively. Therefore, during proteolysis by the protease, the rate of cystathionase inactivation was probably progressively inhibited by the large product accumulated in the medium. This could explain the biphasic time course of inactivation of cystathionase.

Antigenicity of the large molecular weight intermediate product against the specific antibody for intact cystathionase

Previously, we reported that proteolysis of the apo-form of ornithine aminotransferase by the serine protease from the small intestine produced a large molecular weight product which showed no catalytic activity but which retained antigenicity(3). In this work we examined the antigenicity of the large molecular weight intermediate formed during degradation of cystathionase. Cystathionase activity and antigenic reactivity decreased in parallel during proteolysis. Moreover, as shown in Fig. 6, the isolated product did not give a precipitin line with antibody against cystathionase in the Ouchterlony double diffusion test. These results indicate that the large molecular weight intermediate had neither catalytic nor antigenic activity.

Limited proteolysis of ornithine aminotransferase by the protease from the small intestine

Previously we reported(3) that serine protease from the small intestine

Fig. 6. Ouchterlony double diffusion analysis of the large molecular weight intermediate product. The product was separated by applying the reaction products of cystathionase apoenzyme released by the protease to a Sephadex G-100 column, as shown in Fig. 3. Antiserum against cystathionase holoenzyme ($5\mu\text{l}$) was placed in the center well. Samples ($10\mu\text{g}$ protein) of holoenzyme, apoenzyme, intermediate large product and the protease were placed in wells 1–4, respectively.

inactivates the apo-form of ornithine aminotransferase by limited proteolysis, forming a large molecular weight product, which is similar to the native apoenzyme. The molecular weight of the product was measured by Sephadex G-100 column chromatography, and the apparent molecular weights of the native enzyme and the product were calculated to be 67,000 and 60,000–62,000, respectively. No difference was found in the mobilities of the native enzyme and the product on polyacrylamide gel electrophoresis without SDS. However, it was uncertain whether proteolysis resulted in nicks in the apoenzyme. Thus, in this study we followed the proteolysis of ornithine aminotransferase with serine protease by SDS-polyacrylamide gel electrophoresis. Ornithine aminotransferase was incubated with the protease (20:1) at 37°C . Figure 7 shows that during proteolysis disappearance of band A of the subunit (molecular weight 45,000) of the native enzyme was accompanied by the appearance of four distinct new bands of protein, designated as B, C, D and E. Bands B and D were those of intermediates, because during proteolysis they first increased and then disappeared. As they disappeared, bands C and E appeared, and these did not disappear on further incubation with the protease for more than 1 hr. The molecular weights of the products were estimated as described under METHODS (Fig. 8): that of the native subunit, referred to as the A form, was 45,000 and those of the B, C, D and E forms were 41,500, 25,500, 15,000 and 13,500, respectively. These results suggest that during limited proteolysis, the apo-form of ornithine aminotransferase is degraded to intermediate forms B and D and later, to stable forms C and E. Inactivation of the apo-form of ornithine aminotransferase seemed to coincide with decrease in the band of the native enzyme. Therefore, from the results in Fig. 7 and previous data (3) it is concluded that the intermediate forms B

Fig. 7. Kinetics of proteolysis of ornithine aminotransferase by serine protease from the rat small intestine. The apoenzyme of ornithine aminotransferase (2.6 mg/ml) was incubated at 37°C with the protease (4: 1, w/w) in 0.1 M potassium phosphate buffer (pH 8.0). At the times indicated on the abscissa, aliquots were removed for assay of ornithine aminotransferase and 20- μ g samples of protein were analyzed on SDS-acrylamide gels (see METHODS); (●—●) percentage residual activity of ornithine aminotransferase.

Fig. 8. Estimation of the molecular weights of the products of ornithine aminotransferase. Polyacrylamide gels (10% with sodium dodecylsulfate) were used as described under METHODS. The marker proteins were: (1) serum albumin, (2) ovalbumin; (3) glyceraldehyde-3-phosphate dehydrogenase; (4) chymotrypsinogen; (5) myoglobin; (6) cytochrome *c*.

and D have no activity. The intermediate product of ornithine aminotransferase of large molecular weight is formed rapidly in the initial reaction of the serine protease, but the subsequent proteolytic events proceed much more slowly.

DISCUSSION

In the previous paper, it was suggested that the rate of degradation of ornithine transaminase was accelerated in pyridoxine deficiency and that the step of conversion of the holoenzyme to the apoenzyme might be the rate-limiting step in degradation of the enzyme *in vivo* (18). In this paper, we have shown that the higher susceptibility of the apo-form is due to the difference in its tertiary structure from that of the holoenzyme. Serine protease from the small intestine cleaves peptide bonds such as Tyr-Leu, Try-Leu or Phe-Phe of glucagon (19), and thus the increase in susceptibility may be associated with an increase in the number of exposed tryptophan residues in the apoenzyme.

The apo-forms of cystathionase and ornithine aminotransferase were degraded in different ways. The degradation of cystathionase apoenzyme may involve a type of "one by one" attack, in which the first break in the protein molecule is followed by rapid and extensive degradation of the polypeptide chain. On the other hand, the proteolysis of ornithine aminotransferase may proceed according to the zipper-type reaction of LINDERSTRÖM-LANG (20). This difference in the modes of proteolysis of the apo-forms of these two pyridoxal enzymes by serine protease seems to be due to differences in the conformations of the two enzyme molecules and depends on the properties of the large molecular weight products formed initially.

REFERENCES

- 1) KOMINAMI, E., KOBAYASHI, K., KOMINAMI, S., and KATUNUMA, N. (1972): Properties of a specific protease for pyridoxal enzymes and its biological role. *J. Biol. Chem.*, **247**, 6848-6855.
- 2) KATUNUMA, N., KOMINAMI, E., KOBAYASHI, K., BANNO, Y., SUZUKI, K., CHICHIBU, K., HAMAGUCHI, Y., and KATSUNUMA, T. (1975): Studies on new intracellular proteases in various organs of rat. 1. Purification and comparison of their properties. *Eur. J. Biochem.*, **52**, 37-50.
- 3) KOMINAMI, E., BANNO, Y., CHICHIBU, K., SHIOTANI, T., HAMAGUCHI, Y., and KATUNUMA, N. (1975): Studies on new intracellular proteases in various organs of rat. 2. Mode of limited proteolysis. *Eur. J. Biochem.*, **52**, 51-57.
- 4) BANNO, Y., SHIOTANI, T., TOWATARI, T., YOSHIKAWA, D., KATSUNUMA, T., AFTING, E. G., and KATUNUMA, N. (1975): Studies on new intracellular proteases in various organs of rat. 3. Control of group-specific protease under physiological conditions. *Eur. J. Biochem.*, **52**, 59-63.
- 5) MATSUZAWA, T., KATSUNUMA, T., and KATUNUMA, N. (1968): Crystallization of ornithine transaminase and its properties. *Biochem. Biophys. Res. Commun.*, **32**, 161-166.
- 6) SANADA, Y., SHIOTANI, T., OKUNO, E., and KATUNUMA, N. (1976): Coenzyme-dependent conformational properties of rat liver ornithine aminotransferase. *Eur. J. Biochem.*, **69**, 507-515.
- 7) MATSUO, Y., and GREENBERG, D. M. (1958): A crystalline enzyme that cleaves homoserine and cystathionine. *J. Biol. Chem.*, **230**, 545-560.
- 8) MUSHAHWAR, I. K., and KOEPPE, R. E. (1973): Rat liver L-diaminopropionate ammonia lyase,

- identification as cystathionase. *J. Biol. Chem.*, **248**, 7407-7411.
- 9) SAYRE, F. W., and GREENBERG, D. M. (1956): Purification and properties of serine and threonine dehydrases. *J. Biol. Chem.*, **220**, 787-799.
- 10) CHURCHICH, J. E., and DUPOURQUE, D. (1972): Dissociation of cystathionase. *Biochem. Biophys. Res. Commun.*, **46**, 524-530.
- 11) LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. (1951): Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
- 12) WEBER, K., and OSBORN, M. (1969): The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.*, **244**, 4406-4412.
- 13) PASCAL, T. A., TALLAN, H. H., and GILLAM, B. M. (1972): Hepatic cystathionase: Immunochemical and electrophoretic studies of the human and rat forms. *Biochim. Biophys. Acta*, **285**, 48-59.
- 14) CLAUSEN, J. (1969): Immunochemical techniques for the identification and estimation of macromolecules, in *Laboratory Techniques in Biochemistry and Molecular Biology*, ed. by Work, T. S., and Work, E., Inc., North-Holland, Vol. 1, pp. 397-557.
- 15) KHAIRALLAH, E. A., and PITOT, H. C. (1968): Studies on the turnover of serine dehydrase: Amino acid induction, glucose repression, and pyridoxine stabilization, in *Symposium on Pyridoxal Enzymes*, ed. by Yamada, K., Katsunuma, N., and Wada, H., Inc., Maruzen, Tokyo, pp. 159-165.
- 16) KATUNUMA, N. (1973): Enzyme degradation and its regulation by group-specific proteases in various organs of rats. *Curr. Top. Cell. Regul.*, **7**, 175-203.
- 17) LOISELET, J., and CHATAGNER, F. (1966): Amino acid composition of "ethionine-induced" cystathionase of rat liver. *Biochim. Biophys. Acta*, **130**, 180-183.
- 18) KOMINAMI, E., and KATUNUMA, N. (1976): Studies on new intracellular proteases in various organs of rats; participation of protease in degradation of ornithine aminotransferase *in vitro* and *in vivo*. *Eur. J. Biochem.*, **62**, 425-430.
- 19) KOBAYASHI, K., and KATUNUMA, N. (1978): Relative cleavage of peptide bonds by serine protease from the muscle layer of rat small intestine. *J. Biochem.*, **84**, 65-74.
- 20) LINDERSTROM-LANG, K., and MILLER, K. M. (1952): Proteolytic enzymes in proteins and enzymes, in *Lane Medical Lectures, Inc., Stanford Univ., Calif., Vol. 6*, pp. 57-84.