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Effects of Substitutions of the Conserved Histidine Residues in Human γ-Glutamyl Transpeptidase[1]

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γ-Glutamyl transpeptidase possesses two histidine residues at positions 383 and 505 which are conserved in all mammalian and bacterial species. In order to elucidate the functions of these residues, we prepared mutants in which these residues were replaced by Ala. Kinetic analysis of the hydrolysis of L-γ-glutamyl-*p*-nitroanilide indicated that substitution at His-383 decreased the V_{max} value to 14% of that of the wild type, but had no effect on V_{max}/K_m. In reactions involving glycylglycine as the acceptor substrate, the V_{max} value of this mutant decreased to 38% with little alteration of V_{max}/K_m for L-γ-glutamyl-*p*-nitroanilide as a γ-glutamyl donor, but with a significant reduction of V_{max}/K_m for the acceptor. These results show that this substitution causes impairment of the step in which the free enzyme is regenerated from the γ-glutamyl enzyme by water or an acceptor substrate. On the other hand, replacement of His-505 resulted in a decrease of the V_{max} value for transpeptidation to about 10% of that of the wild type despite no substantial effect on the V_{max} value for the hydrolysis reaction. However, this substitution did not affect V_{max}/K_m for the acceptor on transpeptidation. Thus, the formation of a non-productive enzyme-substrate complex with the acceptor substrate would decrease the V_{max} value on transpeptidation. These results suggest that His-383 plays an important catalytic role in facilitating the degradation of the γ-glutamyl-enzyme through hydrolysis or transfer of the γ-glutamyl moiety to an acceptor. It was also shown that His-505 is important in the formation of a complex of the γ-glutamyl enzyme with the acceptor substrate even though it plays no critical role in the catalysis. Although the pH-dependence profile and the van't Hoff plot for the ionic group responsible for enzyme activity were consistent with the requirement of a histidine residue, neither of the conserved histidines could be assigned as such an ionic group. This suggests that another histidine residue(s) might play an essential role in the enzyme function.

Key words: γ-glutamyl transpeptidase, histidine.

γ-Glutamyl transpeptidase plays a critical role in the metabolism of glutathione in mammalian species. The enzyme is a heterodimeric glycoprotein anchored on the surface of the cell membrane, and catalyzes the transfer of a γ-glutamyl group from glutathione and γ-glutamyl compounds to amino acids and dipeptides. When water acts as the acceptor, the reaction leads to hydrolysis (*1, 2*).

Many studies have been carried out to elucidate the reaction mechanism of γ-glutamyl transpeptidase (*3-9*). Chemical modification and site-directed mutagenesis have shown that several specific amino acid residues are obligatory for the creation of an active site (*10-16*). However, the details of the catalytic mechanism of the enzyme remain still unclear. Although a γ-glutamyl-enzyme intermediate is thought to be involved in the reaction of the enzyme (*17*), the catalytic residue γ-glutamylated has not definitely been identified because the crystal structure of the enzyme-substrate complex has not been determined. It has been suggested in some reports that there is a requirement for a hydroxyl residue on the light subunit (*5, 6, 10, 13, 16*). Chemical modification studies involving affinity-labeling reagents showed that the catalytic site of γ-glutamyl transpeptidase is located on the light subunit (*18*). Furthermore, the light subunit isolated from the holo-enzyme exhibits proteolytic activity (*19*), suggesting that in addition to a presumed catalytic nucleophile, a structure necessary to hydrolyze an amide linkage is included in the light subunit.

In many enzymes, a histidine residue is known to serve as an acid-base catalyst facilitating nucleophilic attacks, a typical example being as found in the catalytic triad in serine-class hydrolases (*20*). Likewise, a histidine residue is assumed to function as a catalytic residue in γ-glutamyl transpeptidase by analogy to the formation of an acyl-enzyme intermediate (*17*). Comparison of amino acid sequences of mammalian and bacterial species has shown that γ-glutamyl transpeptidases have two conserved histidine residues, both of which are located on the light subunit (*21, 22*). However, the catalytic roles of these conserved

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histidine residues have not been examined yet.

In the present study we used human γ-glutamyl transpeptidase mutants in which these His residues were replaced by Ala to evaluate their roles in the reaction mechanism.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and DNA modifying enzymes were obtained from New England Biolabs. L-γ-Glutamyl-*p*-nitroanilide, glycylglycine, and other common reagents were purchased from Sigma.

Site-Directed Mutagenesis and Construction of Transfer Plasmids—Amino-acid substitutions were obtained by mutagenesis of the cDNA for human γ-glutamyl transpeptidase (*22*), according to Kunkel (*23*). A 3′ *Apa*I-*Eco*RI fragment of 0.8 kb, which contained the whole light subunit, was subcloned into pBluescript SK+, and then mutations were made using synthetic oligonucleotide primers and the uracil template prepared from the plasmids. The primers used in this study were 5′-GGCACTGCTGCGCTGTCTGTC-3′ for H383A and 5′-CCCGGCTGGCCAACCAGC-3′ for H505A. The 3′ 0.8 kb *Bgl*II-*Eco*RI fragments excised from the resultant plasmids carrying the mutations and the 5′ *Not*I-*Eco*RI 1.0 kb fragment of the wild-type sequence were ligated to the *Not*I and *Eco*RI sites of the transfer vector, pVL1392. A double mutant (H383A/H505A) was prepared by combination of the mutated sequences using the *Bsp*EI site between these mutations. The constructed transfer plasmids were subjected to transfection experiments to generate recombinant baculoviruses.

Cell Culture and General Manipulation of Viruses—*Spodoptera frugiperda* (Sf) 21 cells were maintained at 27°C in Grace's insect media (Gibco-BRL) supplemented with 10% fetal bovine serum, 3.33 g/liter yeastolate, 3.33 g/liter lactalbumin hydrolysate, and 50 mg/liter gentamicin. Recombinant viruses were manipulated as described (*24*).

Preparation of Recombinant Viruses—One microgram of the purified transfer plasmids was used to transfect insect cells with 10 ng of BaculoGold DNA (PharMingen) as a *Autographa Californica* nuclear polyhedrosis viral genome. Transfection experiments were carried out using the Lipofectin (BRL) method (*25*). Media containing recombinant viruses were collected 5 days after transfection, and the generated recombinant viruses were amplified to more than 1×10^7 plaque forming units/ml prior to use.

Expression and Purification of Recombinant Enzymes—Sf21 cells (2×10^8) were infected with the recombinant viruses at a multiplicity of infection of 4. After 80 h incubation the cells were harvested. The expressed proteins were purified as described previously (*14, 16, 26*). The recombinant γ-glutamyl transpeptidases were solubilized by Triton X-100 and papain digestion. The enzymes were further purified by a series of column chromatographic steps including on hydroxylapatite (Bio-Rad), chromatofocusing (PBE94, Pharmacia), and gel filtration (Sephacryl S-200HR, Pharmacia). The purified enzymes gave only two bands, as revealed on silver staining (*27*), and sodium dodecylsulfate-polyacrylamide gel electrophoresis (*28*), corresponding to heavy (44 kDa) and light (24 kDa) subunits in agreement with previous studies (*26*).

Assaying of Enzyme Activity—Assaying was performed at 37°C in 0.1 M Tris-HCl buffer (pH 8.0) using 1 mM L-γ-glutamyl-*p*-nitroanilide as the donor substrate and 20 mM glycylglycine as the acceptor, as previously described (*2*). One unit of activity was defined as the quantity of enzyme that released 1 μmol of *p*-nitroaniline per min at 37°C.

Kinetic Analysis—Transpeptidation activity was assayed at 37°C in 0.1 M Tris-HCl buffer (pH 8.0) using 0.25-2 mM L-γ-glutamyl-*p*-nitroanilide and 5-80 mM glycylglycine, as donor and acceptor substrates, respectively. For assessment of hydrolysis, 6.7-1,280 μM of L-γ-glutamyl-*p*-nitroanilide was used in the absence of glycylglycine. The kinetic parameters for hydrolysis were calculated using the data for substrate ranges in which substrate-activation kinetics did not appear. The release of *p*-nitroaniline was monitored at 410 nm using a Cary 210 spectrophotometer (Varian). Kinetic parameters were calculated by non-linear regression analysis based on the Marquardt algorithm with a curve-fitting computer program (SigmaPlot; Jandel Scientific).

pH-Dependence Study—Hydrolytic activities were determined using 5, 6.7, 10, and 20 μM of L-γ-glutamyl-*p*-nitroanilide in the absence of an acceptor in the pH range of 5.0-8.0. The buffer component (50 mM) used were MES-NaOH for below pH 6.5 and HEPES-NaOH for above pH 6.5. The substrate solutions contained 0.2 M NaCl to avoid the influence of ionic strength differences in the buffer components. The kinetic parameters at each pH value were determined as described above.

Protein Assay—Protein was determined by the bicinchoninic acid method using bovine serum albumin as a standard (*29*).

RESULTS

The wild-type, and H383A and H505A mutant γ-glutamyl transpeptidases were produced using a baculovirus-insect cell expression system, and purified from insect cells infected with recombinant viruses as previously described (*26*). The H383A mutant enzyme, as well as the wild type and H505A ones, was a heterodimer in contrast to the expression of the corresponding bacterial enzyme mutant a single chain form (*30*). The specific activities of the purified enzymes were determined under the standard assay conditions (Table I). Replacement of His-383 and His-505 decreased the activity to 34 and 10% of that of the wild type, respectively.

The kinetic properties of the mutant γ-glutamyl transpeptidases were compared to elucidate the functions of the conserved residues, His-383 and His-505, in the reaction mechanism. Substitution of His-383 led to a reduction of

TABLE I. **Specific activities of the purified enzymes.**

Enzyme	Specific activity[a] (units/mg)
Wild type	440
H383A	150
H505A	47
H383A/H505A	12

[a]Activity was assayed at 37°C with 1 mM L-γ-glutamyl-*p*-nitroanilide as the donor substrate and glycylglycine as the acceptor in 0.1 M Tris-HCl buffer (pH 8.0).

V_{max} for the hydrolysis of L-γ-glutamyl-*p*-nitroanilide to 38% of that of the wild type (Table II). However, the mutation resulted in no significant change of V_{max}/K_m, as shown by the parallel lines in double reciprocal plots for the wild type and the H383A mutant (Fig. 1). The hydrolysis of a γ-glutamyl substrate by γ-glutamyl transpeptidase is believed to proceed *via* a γ-glutamyl enzyme, as follows.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E' \xrightarrow{k_3} E + P_2 \qquad (P_1 \text{ released at } k_2)$$

Here, E, S, ES, and E′ indicate the free enzyme, γ-glutamyl substrate, Michaelis complex, and γ-glutamyl enzyme, respectively. P_1 corresponds to the group leaving the γ-glutamyl substrate, and P_2 is glutamate. For the steady state kinetics of this reaction scheme, the parameters are defined as follows: $V_{max} = k_2k_3E_0/(k_2+k_3)$, where E_0 is the total concentration of the enzyme, $K_m = k_3(k_{-1}+k_2)/\{k_1(k_2+k_3)\}$, and $V_{max}/K_m = k_1k_2E_0/(k_{-1}+k_2)$. Therefore, the effects of the replacements on the kinetic parameters may be explained by the reduction of only the rate constant, k_3, which controls the rate of degradation of the γ-glutamyl enzyme or regeneration of the free enzyme from an acyl enzyme species. Thus, the apparent decrease in the K_m value would not be caused by the higher affinity for the substrate but probably the accumulation of a γ-glutamyl enzyme species. These results suggest that His-383 is involved in cleavage of the γ-glutamyl linkage in the γ-glutamyl enzyme but not necessary for binding of the donor substrate or formation of the γ-glutamyl enzyme.

On transpeptidation by the H383A mutant with glycylglycine as an acceptor, kinetic properties similar to those seen on hydrolysis were observed for the donor substrate, L-γ-glutamyl-*p*-nitroanilide. V_{max}/K_m for the donor was almost of the same order as for the wild-type enzyme, whereas the value for the acceptor was less than 10% (Table II). This is also consistent with the involvement of His-383 in degradation of the γ-glutamyl enzyme through the transfer of the γ-glutamyl moiety to the acceptor. In addition, replacement of His-383 gave a 4-fold increased K_m value for the acceptor, although no increase in K_m for the donor was observed. His-383 might be associated functionally with an acceptor-subsite of the active center.

Substitution at His-505 did not significantly decrease V_{max} for hydrolysis (Fig. 1 and Table III), indicating that it plays no major catalytic role in this reaction. On transpeptidation, however, the V_{max} value was markedly lowered (Table II). Thus, the mutation at His-505 appeared to only abolish the reaction with the acceptor substrate, *i.e.* it did not impair the hydrolytic reaction. The decrease in V_{max} on transpeptidation with much lower change in the V_{max}/K_m value for the acceptor substrate suggests the formation of a non-productive complex of the enzyme with the acceptor substrate (*31*). In such a complex, the acceptor substrate would occupy the active site but in a manner that does not involve the usual reaction. In contrast to the mutation of His-383, substitution at His-505 seems to affect the binding of L-γ-glutamyl-*p*-nitroanilide, as indicated by the increase in K_m for the hydrolysis reaction. Although the K_m value for the donor substrate on transpeptidation can decrease, as well as that for the acceptor on the formation of a non-productive acceptor complex, the decrease in the binding of the γ-glutamyl donor due to the mutation would be counteracted by a possible decrease in K_m for the donor.

A double mutant as to His at residues 383 and 505 was also produced to examine the possibility that one of these residues functions in the absence of the other. The activity assay showed that about 2.7% of the activity of the wild type still remained even after replacement of both histidines (Table I). Therefore, it is clearly indicated that conservation of both imidazole groups is not absolutely essential. The results obtained on kinetic analyses may be interpreted as reflecting the additive effect of single

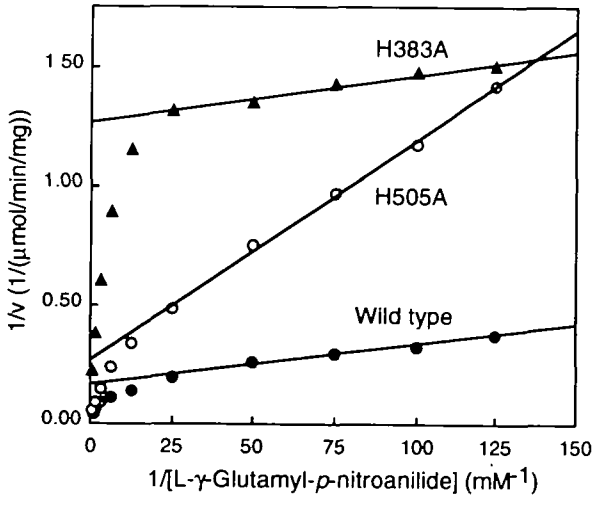

Fig. 1. **Double reciprocal plots of the hydrolysis of L-γ-glutamyl-*p*-nitroanilide by the wild-type and mutant γ-glutamyl transpeptidases.** The substrate activation observed with higher concentrations of the substrate is due to the occurrence of an autotranspeptidation reaction in which the γ-glutamyl substrate acts as an acceptor.

TABLE III. **Kinetic parameters for hydrolysis.**

Enzyme	V_{max} (μmol/min/mg)	K_m (μM)	V_{max}/K_m (μmol/min/mg/mM)
Wild type	5.5±0.17	8.1±0.64	680
H383A	0.79±0.009	1.6±0.17	490
H505A	3.2±0.13	28±1.7	110
H383A/H505A	0.12±0.002	8.1±0.31	15

TABLE II. **Kinetic parameters for transpeptidation activity.**

Enzyme	V_{max} (μmol/min/mg)	γ-Glutamyl-*p*-nitroanilide		Glycylglycine	
		K_m (mM)	V_{max}/K_m (μmol/min/mg/mM)	K_m (mM)	V_{max}/K_m (μmol/min/mg/mM)
Wild type	1,200±30	1.4±0.05	860	10±0.6	120
H383A	450±34	0.70±0.02	640	41±5.2	11
H505A	130±13	1.3±0.06	100	2.1±0.90	62
H383A/H505A	35±1.8	1.5±0.04	23	8.4±1.25	4.2

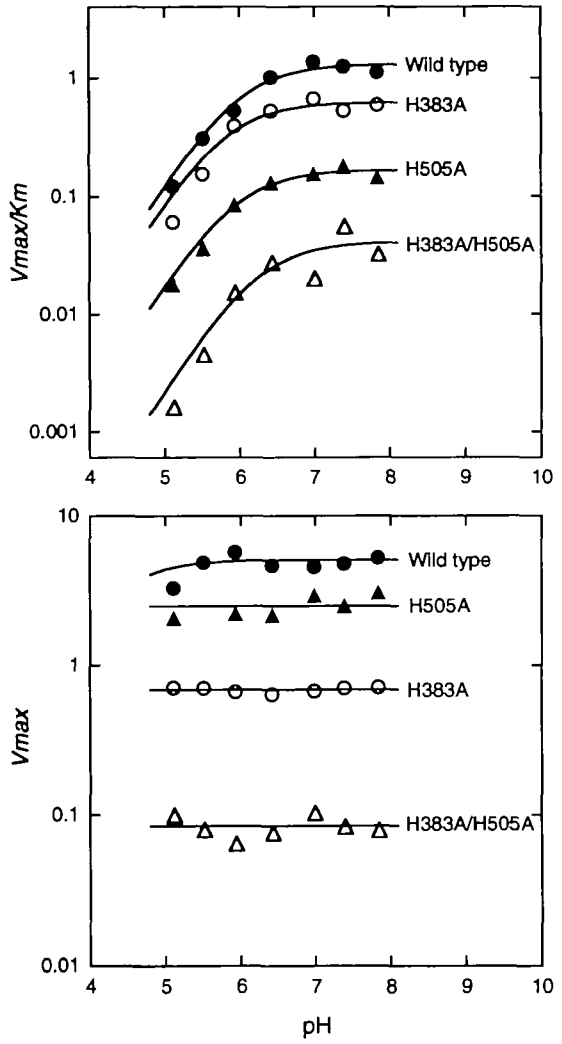

Fig. 2. **pH-Dependence profiles of the V_{max}/K_m and V_{max} values for the hydrolysis of L-γ-glutamyl-*p*-nitroanilide by the wild-type and mutant enzymes.** The kinetic values are expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1} \cdot \mu M^{-1}$ and $\mu mol \cdot min^{-1} \cdot mg^{-1}$ for V_{max}/K_m and V_{max}, respectively.

mutations.

In order to determine whether or not the conserved imidazole groups are critical ionic groups, the pH-dependence of the kinetic parameters on the hydrolysis of L-γ-glutamyl-*p*-nitroanilide was investigated. As shown by the pH-dependence profile for V_{max}/K_m of the wild type (Fig. 2), an ionic group with a pK_a of approximately 6 is responsible for the activity, while no obvious pH-dependence of V_{max} was observed. Furthermore, the enthalpy change for this ionization was demonstrated to be 5.4 kcal/mol by the van't Hoff plot of pK_a for V_{max}/K_m of the wild-type enzyme (Fig. 3). Hence, the responsible ionic group is most likely an imidazole (*32*). Nevertheless, none of the mutations virtually affected the dependence profile, indicating that neither His-383 nor His-505 can be assigned as this important ionic residue.

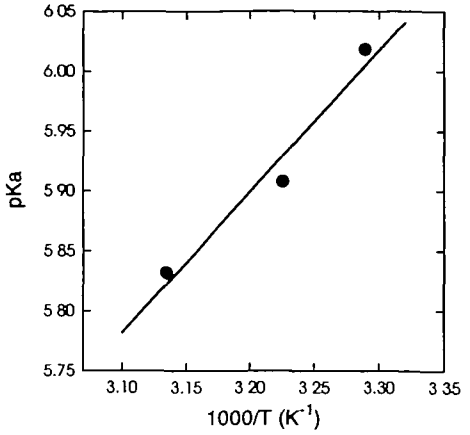

Fig. 3. **van't Hoff plot of the pK_a values for V_{max}/K_m for the hydrolysis of L-γ-glutamyl-*p*-nitroanilide by the wild-type γ-glutamyl transpeptidase.**

DISCUSSION

On the basis of the analogy between the reactions catalyzed by γ-glutamyl transpeptidase and serine-class hydrolases it appears that a histidine residue plays a role as an acid-base catalyst (*20*). We have carried out site-directed mutagenesis of the conserved histidine residues, which are the most likely candidates that activate the presumed catalytic hydroxyl group responsible for the formation of a γ-glutamyl enzyme intermediate and for its deacylation. In general, a catalytically essential residue such as a constituent of the catalytic triad found in serine proteases should be conserved within a family of enzymes and the loss of such a residue should result in marked modification of the enzyme activity.

However, the replacement of His-383 or His-505 in the human enzyme only lowered its activity, and the mutant enzyme with both residues substituted still exhibited activity. These results indicate that these residues are not obligatory for the activity of human γ-glutamyl transpeptidase. Furthermore, since the pH-dependence profile of the double mutant was quite similar to that of the wild type, neither of the conserved histidine residues appears to participate critically as an acid-base catalyst in the formation of the γ-glutamyl enzyme. The unidentified ionic group revealed by the pH profile may be assigned to another imidazole group, which is not conserved among species, because the van't Hoff plot of the pH-dependence of V_{max}/K_m for the wild-type enzyme gave an enthalpy change of 5.4 kcal/mol for this ionization. This enthalpy change and the pK_a value around 6.0 indicate that an imidazole plays an important role in the catalytic activity. It is also possible that a carboxyl or amino group located in an unusual environment might exhibit an unexpected pK_a. The identification of this important ionic group responsible for activity is required.

The present study showed that His-383 and -505 are required for a fully active enzyme. Since His-383 functions to regenerate the free enzyme, it is likely that this residue partially assists in the nucleophilic attack by water or an acceptor substrate against the carbonyl moiety of the γ-glutamyl enzyme. In this respect, His-383 could serve as

a weak acid-base catalyst in disruption of the γ-glutamyl bond in the acyl enzyme. However, this possible function does not appear to be essential because water and an amino group of the acceptor would be sufficiently nucleophilic. Alternatively, the kinetic properties of the mutant could also be explained by hindrance of the breakdown of the enzyme-product complex. On the other hand, since the mutation at His-505 appeared to cause the formation of a non-productive complex with the acceptor, the residue in question would be required to orient the acceptor substrate in the active site so as to permit the substrate to react with the γ-glutamyl enzyme. However, this residue does not appear to be involved in the reaction of water with the acyl enzyme.

If γ-glutamyl transpeptidase employs a hydroxyl group as a catalytic nucleophile to form an acyl-enzyme intermediate, the enzyme would require an acid-base catalyst to increase the nucleophilicity of the presumed catalytic hydroxyl group. The present study suggests that either another imidazole or another kind of group might act as the catalyst. The assignment of the ionic group revealed by the pH-dependence profile could lead to identification of this catalyst.

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REFERENCES

1. Tate, S.S. and Meister, A. (1981) γ-Glutamyl transpeptidase: Catalytic, structural and functional aspects. *Mol. Cell. Biochem.* **39**, 357-368
2. Tate, S.S. and Meister, A. (1985) γ-Glutamyl transpeptidase from kidney. *Methods Enzymol.* **113**, 400-419
3. Szewczuk, A. and Connell, G.E. (1965) The reaction of iodoacetamide with the active center of γ-glutamyl transpeptidase. *Biochim. Biophys. Acta* **105**, 352-367
4. Inoue, M., Horiuchi, S., and Morino, Y. (1977) Affinity labeling of rat-kidney γ-glutamyl transpeptidase. *Eur. J. Biochem.* **73**, 335-342
5. Tate, S.S. and Meister, A. (1978) Serine-borate complex as a transition state inhibitor of γ-glutamyl transpeptidase. *Proc. Natl. Acad. Sci. USA* **75**, 4806-4809
6. Inoue, M., Horiuchi, S., and Morino, Y. (1978) Inactivation of γ-glutamyl transpeptidase by phenylmethanesulfonyl fluoride, a specific inactivator of serine enzymes. *Biochem. Biophys. Res. Commun.* **82**, 1183-1188
7. Reed, D.J., Ellis, W.W., and Meck, R.A. (1980) The inhibition of γ-glutamyl transpeptidase and glutathione metabolism of isolated rat kidney cells by L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125; NSC-163501). *Biochem. Biophys. Res. Commun.* **94**, 1273-1277
8. Elce, J.S. (1980) Active-site amino acid residues in γ-glutamyltransferase and the nature of the γ-glutamyl-enzyme bond. *Biochem. J.* **185**, 473-481
9. Stole, E., Smith, T.K., Manning, J.M., and Meister, A. (1994) Interaction of γ-glutamyl transpeptidase with acivicin. *J. Biol. Chem.* **269**, 21435-21439
10. Stole, E., Seddon, A.P., Wellner, D., and Meister, A. (1990) Identification of a highly reactive threonine residue at the active site of γ-glutamyl transpeptidase. *Proc. Natl. Acad. Sci. USA* **87**, 1706-1709
11. Stole, E. and Meister, A. (1991) Interaction of γ-glutamyl transpeptidase with glutathione involves specific arginine and lysine residues of the heavy subunit. *J. Biol. Chem.* **266**, 17850-17857
12. Ikeda, Y., Fujii, J., and Taniguchi, N. (1993) Significance of Arg-107 and Glu-108 in the catalytic mechanism of human γ-glutamyl transpeptidase: Identification by site-directed mutagenesis. *J. Biol. Chem.* **268**, 3980-3985
13. Smith, T.K., Ikeda, Y., Fujii, J., Taniguchi, N., and Meister, A. (1995) Different sites of acivicin binding and inactivation of γ-glutamyl transpeptidases. *Proc. Natl. Acad. Sci. USA* **92**, 2360-2364
14. Ikeda, Y., Fujii, J., Taniguchi, N., and Meister, A. (1995) Human γ-glutamyl transpeptidase mutants involving conserved aspartate residues and the unique cysteine residue of the light subunit. *J. Biol. Chem.* **270**, 12471-12475
15. Smith, T.K. and Meister, A. (1995) Chemical modification of active site residues in γ-glutamyl transpeptidase: Aspartate 422 and cysteine 453. *J. Biol. Chem.* **270**, 12476-12480
16. Ikeda, Y., Fujii, J., Anderson, M.E., Taniguchi, N., and Meister, A. (1995) Involvement of Ser-451 and Ser-452 in the catalysis of human γ-glutamyl transpeptidase. *J. Biol. Chem.* **270**, 22223-22228
17. Allison, R.D. (1985) γ-Glutamyl transpeptidase: Kinetics and mechanism. *Methods Enzymol.* **113**, 419-437
18. Tate, S.S. and Meister, A. (1977) Affinity labeling of γ-glutamyl transpeptidase and location of the γ-glutamyl binding site on the light subunit. *Proc. Natl. Acad. Sci. USA* **74**, 931-935
19. Gardell, S.J. and Tate, S.S. (1979) Latent proteinase activity of γ-glutamyl transpeptidase light subunit. *J. Biol. Chem.* **254**, 4942-4945
20. Blow, D.M., Birktoft, J.J., and Hartley, B.S. (1969) Role of a buried acid group in the mechanism of action of chymotrypsin. *Nature* **221**, 337-340
21. Suzuki, H., Kumagai, H., Echigo, T., and Tochikura, T. (1989) DNA sequence of the *Escherichia coli* K-12 γ-glutamyltranspeptidase gene, *ggt*. *J. Bacteriol.* **171**, 5169-5172
22. Sakamuro, D., Yamazoe, M., Matsuda, Y., Kangawa, K., Taniguchi, N., Matsuo, H., Yoshikawa, H., and Ogasawara, N. (1988) The primary structure of human γ-glutamyl transpeptidase. *Gene* **73**, 1-9
23. Kunkel, T.A. (1985) Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* **82**, 488-492
24. Piwnica-Worms, H. (1987) Expression of proteins in insect cells using baculovirus vectors in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, eds.) pp. 16.8.1-16.11.7, Greene Publishing Associates and Wiley-Interscience, New York
25. Felgner, P.L., Gadek, T.R., Holm, H., Roman, R., Chan, H.W., Wenz, M., Northrop, J.R., Ringold, G.M., and Danielsen, M. (1987) Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA* **84**, 7413-7417
26. Ikeda, Y., Fujii, J., Taniguchi, N., and Meister, A. (1995) Expression of an active glycosylated human γ-glutamyl transpeptidase mutant that lacks a membrane anchor domain. *Proc. Natl. Acad. Sci. USA* **92**, 126-130
27. Oakley, B.R., Kirsch, D.R., and Morris, N.R. (1980) A simplified silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* **105**, 361-363
28. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **227**, 680-685
29. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985) Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85
30. Hashimoto, W., Suzuki, H., Yamamoto, K., and Kumagai, H. (1995) Effects of site-directed mutations on processing and activity of γ-glutamyltranspeptidase of *Escherichia coli* K-12. *J. Biochem.* **118**, 75-80
31. Fersht, A. (1984) The basic equations of enzyme kinetics in *Enzyme Structure and Mechanism*, 2nd ed., pp. 98-120, W.H. Freeman and Company, New York
32. Hamaguchi, K. (1992) Physicochemical properties of amino acid side chains in *The Protein Molecule: Conformation, Stability and Folding*, pp. 1-20, Springer-Verlag, Tokyo