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Review

Glutathione metabolism in *Escherichia coli* [1]

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Abstract

Glutathione is the most abundant low-molecular-weight thiol compound in aerobic bacterial cells. Although its biosynthetic pathway in *Escherichia coli* is known, its degradative pathway is not clear. We have studied its degradative pathway using *E. coli* K-12 as a model bacterium. Glutathione synthesized during the exponential phase of growth is excreted into the medium. During the stationary phase, extra cellular glutathione penetrates into the periplasm where its γ-glutamyl residue is cleaved off by γ-glutamyltranspeptidase localized in the periplasm. The released cysteinylglycine is taken up into the cytoplasm through peptide transport systems and the peptide linkage of cysteinylglycine is cooperatively cleaved by enzymes with cysteinylglycinase activity. The resultant cysteine and glycine are used as cysteine and glycine sources, respectively. This cycle acts as a salvage system for cysteine (glycine) in the cells. γ-Glutamyltranspeptidase, the key enzyme of this cycle, was studied extensively not only from a physiological point of view, but also with the aim of applying this enzyme as a catalyst for the synthesis of useful γ-glutamyl compounds. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Escherichia coli*; Glutathione; γ-Glutamyltranspeptidase; Cysteinylglycinase; Aminopeptidase; Peptidase B

1. Introduction

Glutathione (GSH) is a tri-peptide with the structure of L-γ-glutamyl-L-cysteinylglycine. It is present in cells of bacteria to mammals. It protects cells not only as an antioxidant, but also binds to reactive electrophilic compounds, and its *S*-conjugates are converted to the corresponding mercapturic acids via the mercapturic acid pathway for excretion from the cells [1,2]. *Escherichia coli* was shown to possess the two enzymes required for GSH synthesis and to accumulate high concentrations of GSH in its cells under aerobic conditions [3]. Genetic engineering of the *E. coli* B strain, which produces a large amount of GSH, was performed [4]. Nevertheless, *E. coli* was thought to have little or no γ-glutamyltranspeptidase (GGT) [5,6], the key enzyme of GSH degradation in mammals [7]. We investigated the growth conditions of *E. coli* K-12 and found that it produces a fairly large amount of GGT in its periplasmic space when grown at 20°C [8]. Since then, we have studied the GSH degradation pathway of bacteria using *E. coli* K-12 as a model bacterium to gain insight into its physiological role and have

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[1] Dedicated to Professor Hideaki Yamada in honor of his 70th birthday.

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also studied the structure–function relationship of its enzymes.

2. Properties of GGT from *E. coli* K-12

E. coli GGT was purified to electrophoretic homogeneity [9]. It consists of two subunits (MW 39,000 and 20,000) and catalyzes two types of reactions: (1) hydrolysis, in which the γ-glutamyl linkage in various γ-glutamyl compounds is hydrolyzed, and (2) transpeptidation, in which the γ-glutamyl moiety is transferred to an amino acid or a peptide.

$$\gamma - \mathrm{Glu} - \mathrm{X} + \mathrm{H_2O} \rightarrow \mathrm{Glu} + \mathrm{XH} \quad (1)$$

$$\gamma - \mathrm{Glu} - \mathrm{X} + \mathrm{AH} \rightarrow \gamma - \mathrm{Glu} - \mathrm{A} + \mathrm{XH} \quad (2)$$

(X, A: amino acid or peptide)

It prefers basic and aromatic amino acids as acceptors of the γ-glutamyl moiety and utilizes L-glutamine as a good donor as glutathione [9].

3. Elucidation of the physiological role of GGT in *E. coli* cells using GGT-deficient mutants

A patient with an inborn deficiency of GGT resulting in glutathionuria has been described [10]. Glutathionuria developed in mice whose GGT was inhibited by inhibitors [11,12], and GGT-deficient mice exhibited both glutathionuria and glutathionanemia [13,14]. These results indicate that the concentration of extra cellular glutathione increases when eukaryotic cells lose their GGT activity. To determine if this is also the case for bacterial cells, we examined a GGT-deficient mutant of *E. coli* K-12. The wild-type strain, MG1655, was mutagenized with ethyl methanesulfonate, followed by screening of GGT-deficient mutants [15]. Two GGT-deficient mutants which had lost the GGT activity completely were chosen and the mutation was named *ggt*. Genetic mapping of *ggt* localized it at about 76 min on the *E. coli* K-12 genome. Following completion of the *E. coli* K-12 genome project, it was remapped at 77.2 min or the 3.58 Mb coordinate [16]. *E. coli* accumulates GSH in the culture medium during the exponential phase and the concentration of it reaches a maximum in the early stationary phase, but thereafter, it decreases rapidly, whereas the amount of GSH in the culture medium decreases much more gradually in the case of a GGT-deficient strain than in the wild-type strain [15]. This suggested that GSH excreted into the medium is utilized by GGT. Since GGT of *E. coli* catalyzes the hydrolysis of various γ-glutamyl compounds [9], we predicted that *E. coli* can utilize an exogenous γ-glutamyl peptide as an amino acid source. One of the most abundant γ-glutamyl peptides existing in nature is GSH. A cysteine auxotroph and a glycine auxotroph were examined to determine if they could utilize GSH as a cysteine source and a glycine source, respectively (Table 1) [17]. The cysteine auxotroph grew on M9 glucose plates supplemented with GSH as well as with cysteine, cysteinylglycine or γ-glutamylcysteine. However, a *ggt* derivative of the cysteine auxotroph did not grow on M9 glucose plates supplemented with GSH or γ-glutamylcysteine, while it grew on ones supplemented with cysteine or cysteinylglycine. Similar results were obtained in the case of the glycine auxotroph.

Table 1
Utilization of glutathione and related compounds by cysteine auxotrophs[a]

Strain	Genotype	M9 glucose medium	+Cys	+γ-Glu–Cys	+Cys–Gly	+GSH	LB
SH786	*cysG*	–	+	+	+	+	+
SH788	*cysG ggt-1*	–	+	–	+	–	+

[a] Plates were incubated for 2 days at 37°C.

These results indicate that *E. coli* can utilize exogenous GSH as a cysteine source and a glycine source, and that GGT is essential for this. One of the important physiological roles of GGT in *E. coli* could be the catalysis of the initial step of salvage or recycling of cysteine. It was also shown in a mammalian cell line and in yeast that GGT catalyzes the initial step of the cleavage of extra cellular GSH, for use as a cysteine source and a nitrogen source, by other researchers [18,19].

As regards the transpeptidation reaction of bacterial GGT, Hara et al. [20] reported that the synthesis of poly-γ-glutamic acid (PGA) by *Bacillus natto* Asahikawa and its GGT activity depended on its plasmid, pUH1, and suggested that GGT was associated with PGA production. *B. natto* produced PGA, which is the main component of the sticky substance of 'natto', that is, fermented soybeans. They determined the nucleotide sequence of an open reading frame (ORF) in pUH1, which they suggested encodes GGT [21]. The amino acid sequence they deduced from that nucleotide sequence exhibits no similarity with the amino acid sequences of GGTs known to date. It does not even contain the N-terminal amino acid sequences of the two subunits obtained on protein sequencing of the purified GGT from *B. natto* by Ogawa et al. [22], nor does it contain the Thr–X–His consensus sequence of GGT found at the N-terminal of the small subunit [see below]. Moreover, the deduced molecular weight of only 49,356 is less than that of known GGT proteins, i.e., ~60,000. Therefore, we think that this ORF does not encode GGT. In their later publications, Hara et al. claim that this ORF encodes PGA-production stimulating factor [23] and may not encode the GGT protein [24], although a transformant of the *B. subtilis* strain with a derivative of plasmid pUH1 produced much more 'GGT activity' than the parent strain [23]. We do not know whether or not the 'GGT activity' they observed was the transpeptidation activity of GGT, because the method they used to assay GGT activity [25] might have only measured the hydrolysis activity of the γ-glutamyl linkage. In contrast with Hara et al., Koehler and Thorne [26] reported that there is no relation between pLS19 (same as pUH1) and PGA production by *B. natto*. More recently, Nagai et al. cured plasmids pUH1 and pNAGL1 from *B. natto* Asahikawa, and found that the cured strain produced PGA of the same quantity and chemical properties as that produced by the parent strain, and discussed that the ORF whose nucleotide sequence Hara et al. determined encodes an analogue of the Mob/Pre protein [27]. As for PGA, it has been shown that capsules of *B. anthracis* [28] and *B. licheniformis* [29] are made from poly-γ-D-glutamic acid. The capsule of *B. anthracis* is one of the important virulence factors of this bacterium [30], which is encoded by *cap* genes on a 60 MDa plasmid [31–33]. Makino et al. cloned *cap* genes [33], and the nucleotide sequences of the *cap* genes were determined [34]. A GGT-deficient mutant of *E. coli* K-12 was transformed with a plasmid containing the *B. anthracis* cap genes and it was found that the *cap* genes could not complement the GGT deficiency, but that the transformant synthesized an immunologically identical capsular peptide [34]. Moreover, the capsular synthesis by this transformant was not inhibited by the GGT-specific inhibitor, 6-diazo-5-oxo-norleucine [34]. These results indicate that none of the *cap* genes products is GGT and that GGT has no relation with the capsule synthesis by *B. anthracis*. It is also known that the capsule of *B. licheniformis* is synthesized by its membrane-associated poly-γ-D-glutamyl synthetase complex [35], while GGTs have been found and purified from extra cellular fractions of *Bacillus* [22,36–38]. Furthermore, we have tried to synthesize PGA in vitro with *E. coli* GGT using L-glutamine as a substrate and found that it is quite difficult to polymerize more than three glutamic acid residues [unpublished result]. From all these results, we conclude that GGT has no relation with the synthesis of PGA by bacteria. In *E. coli*, the transpeptidation activity was found to

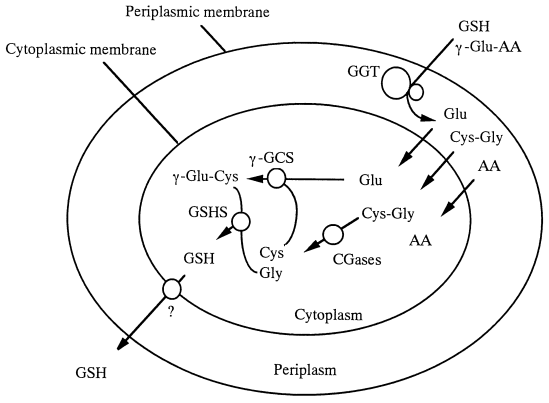

Fig. 1. Glutathione metabolism in *E. coli*. GGT, γ-glutamyltranspeptidase; GSH, glutathione; AA, amino acids; CGase, cysteinylglycinase; γ-GCS, γ-glutamylcysteine synthetase; GSHS, glutathione synthetase.

be negligible when the GGT activity of intact cells was measured [unpublished result]. Besides, since the K_m values for γ-glutamyl acceptors were extremely high [9], it is hard to imagine that the transpeptidation reaction of GGT has any physiological significance in *E. coli*.

4. Cysteinylglycinase of *E. coli* K-12

As described above, GSH was cleaved by GGT located in the periplasmic space to generate glutamate and cysteinylglycine, and the resultant cysteinylglycine was cleaved by enzyme(s) with cysteinylglycinase activity into cysteine and glycine to be utilized as a cysteine source and a glycine source, respectively. Although there are several peptidases in *E. coli* known [39], it has never been reported whether these peptidases can cleave the peptide bond between cysteine and glycine of cysteinylglycine or there is an unknown cysteinylglycinase. We compared the cysteinylglycinase activities of several strains deficient in aminopeptidases and dipeptidase with broad substrate specificities, and found that cytoplasmic aminopeptidases A, B and N, and dipeptidase D are cooperatively responsible for this activity [paper in preparation] (Fig. 1). Among these peptidases, the gene of aminopeptidase B, which is the least known peptidase, was cloned, and it was suggested to be a homohexameric metal peptidase exhibiting high homology with leucine aminopeptidases of mammals and aminopeptidase A of *E. coli* K-12 [40].

5. Features of the *ggt* gene and the subunit structure of GGT

The *E. coli ggt* gene, which encodes GGT, was cloned [41] from the pLC plasmid in the Clarke–Carbon colony bank, its nucleotide sequence was determined and the amino acid sequence of GGT was predicted [42]. The N-terminal signal peptide consists of 25 amino acid residues, the large subunit of 365 residues and the small subunit of 190 residues, which are encoded by a single open reading frame in this order, this being a very rare gene construct (Fig. 2). This suggests that besides the cleavage by signal peptidase I, *E. coli* GGT is subjected to post-translational cleavage between Gln-390 and Thr-391 to yield a heterodimer, as are native mammalian GGTs. Each subunit of *E. coli* GGT was isolated by HPLC and its molecular weight was determined with an ion spray mass spectrometer [43]. The results showed that GGT, unlike penicillin acylase [44], does not lose any

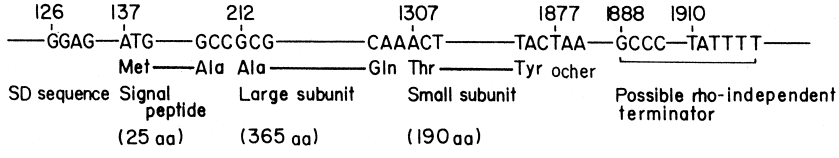

Fig. 2. Structure of the *E. coli ggt* gene.

amino acid residues during the processing. Sequence alignment for similarity at the processing site among GGTs and related enzymes is shown in Fig. 3. Gln-390, the C-terminal amino acid residue of the large subunit, is not conserved, while Thr-391 and His-393, the N-terminal amino acid residues of the small subunit, are conserved among all GGTs whose amino acid sequences have been determined. Periplasmic fractions of the T391A and H393G mutants, having threonyl residue-391 substituted with an alanyl residue and histidinyl residue-393 with a glycine residue, respectively, did not give bands corresponding to the large (39 kDa) or small (20 kDa) subunit on SDS-PAGE gel followed by Western blot analysis, whereas they gave a new 59 kDa band (Fig. 4) [45]. This indicates that pro-GGT, which was not processed into two subunits, was accumulated in these mutants. Therefore, the Thr–X–His sequence of the N-terminal of the small subunit is critical for the processing. As can be seen in Fig. 4, periplasmic fractions of T391A, T391S, T392A and Q390A gave a 48 kDa band. This protein might be derived from the precursor through abnormal proteolysis because of the mutations. The intensities of the large and small subunits varied, as shown in Fig. 4. Since we loaded the same amount of protein of each periplasmic fraction, the intensity reflects the expression level and stability of each mutant GGT. In mammals, it has been hypothesized that GGT is processed by a membrane-bound trypsin-like serine protease [46], but there has been no further report about this protease. Recently, from the results of three-dimensional structural analysis, it was suggested that the side chain of the N-terminal amino acid residue of the small subunit (Thr-391 in *E. coli* GGT) is the nucleophilic atom which attacks the carbonyl carbon of the peptide linkage between Gln-390 and Thr-391, and that the processing takes place autocatalytically [47]. However, no experimental proof has been obtained.

On sequence alignment for similarity, we also found a stretch in *E. coli* GGT (amino acid residue numbers 451 to 489) that exhibits especially high similarity with all GGTs whose amino acid sequences have been determined.

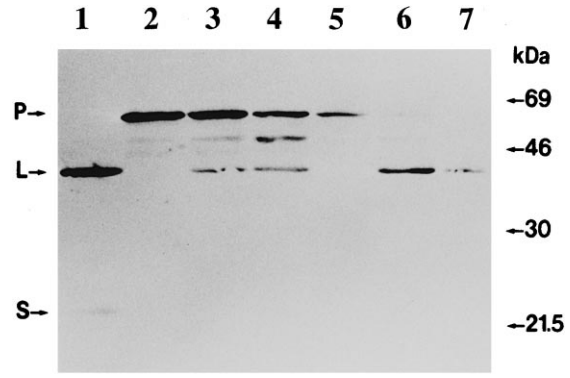

Fig. 4. Effects of mutations around the processing site. A periplasmic fraction of each mutant (20 μg-protein) was separated on an SDS-PAGE gel, followed by Western blot analysis. Lane 1, wild-type; 2, T391A; 3, T391S; 4, T392A; 5, H393G; 6, Q390A; 7, V396T. L, S, and P indicate large subunit, small subunit, and pro-GGT, respectively.

	391
Escherichia coli GGT	ESNQTTHYSVVDK
Pseudomonas A14 GGT	EGSNTTHYSIVDK
Bacillus subtilis GGT*	QEGQTTHFTVTDR
Bacillus subtilis GGT**	VEGQTTHFTVADR
Bacillus natto NR-1 GGT	TTHFTVADR
Mycobacterium tuberculosis GGT	PEHGTSHLSVVDS
Helicobacter pylori GGT	EGSNTTHYSVADR
Synechocystis sp. GGT	KLGSTTHISVLDG
Saccharomyces cerevisiae GGT	NPHGTAHFSIVDS
Caenorhabditis elegans GGT	PDHGTSHVSAMDQ
rat GGT	DDGGTAHLSVVSE
human GGT	DDGGTAHLSVVAE
porcine GGT	DDAGTAHLSVVSD
mouse GGT	DDGGTAHLSAVSE
Arabidopsis thaliana GGT	DDHGTSHLSIIDR
human GGT-related enzyme	HGTGTSHVSVLGE
Pseudomonas sp. AcyI	ESADTTHVTVADA
Bacillus anthracis Dep	EHESTTHFVIIDR
Streptomyces coelicolor transferase	TRGDTCHLDVVDR

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** by K. Xu and M. A. Strauch

Fig. 3. Sequence alignment for similarity at the processing site among GGTs and related enzymes. The arrow indicates the post-translational processing site. Highly conserved amino acid residues are shaded. The number 391 indicates that this Thr residue is at position 391 in *E. coli* GGT.

Therefore, we predict that this region is essential for the enzymatic function of GGT. On the other hand, from the results of chemical modification studies, some basic amino acid residues of mammalian GGTs were suggested to be located in the active center for the enzymatic reaction [48,49]. Therefore, basic amino acid residues which are highly conserved among GGTs (Arg-513 and Arg-571 in *E. coli*) were mutagenized by site-directed mutagenesis, and the effects were observed [50]. Although these residues are far from the processing site in the primary structure of GGT, these mutants did not undergo processing and did not exhibit enzymatic activity. This suggests that the processing occurs after the conformation of GGT has been formed. The amino acid residues in the C-terminal of the B-chain of penicillin acylase, which is subjected to similar post-translational processing, are critical [51,52]. This is one piece of evidence that GGT is processed in a similar way to penicillin acylase, which is one of the proposed N-terminal nucleophile hydrolase superfamily [47].

Kabanov et al. [53] reported that either of the two subunits of mammalian GGT possessed enzymatic activity. Since this conflicted with the results previously obtained for mammalian GGT by Gardell and Tate [54], and our idea, we proved that both subunits of *E. coli* GGT are necessary for its enzymatic activity [43]. The large and small subunits of *E. coli* GGT were separated by HPLC. It was found that neither of the subunits possessed enzymatic activity itself, while the GGT reconstituted from the separated subunits recovered the enzymatic activity (Fig. 5). Moreover, two kinds of plasmid encoding the signal peptide and either the large or small subunit were constructed. A GGT-deficient mutant of *E. coli* was transformed with each plasmid or with both of them. The strain harboring the plasmid encoding one of the subunits produced the corresponding subunit protein in the periplasmic space, but exhibited no enzymatic activity. The strain transformed with both plasmids exhibited enzymatic activity even though

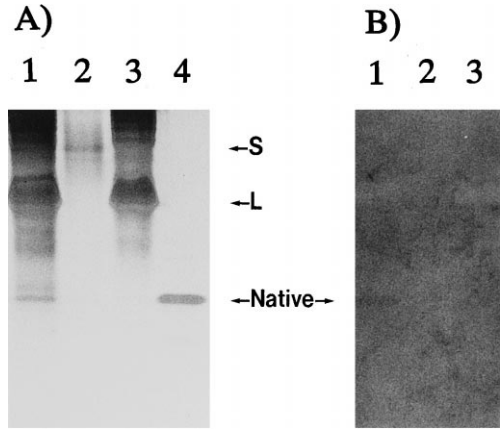

Fig. 5. Analysis of reconstituted GGT by native PAGE. (A) Silver staining, (B) GGT-activity staining. Lane 1, large subunit (49 μg) and small subunit (45 μg) incubated in 20 mM Tris–HCl (pH 8.3) at 4°C for 15 h; 2, only the small subunit was incubated as above (45 μg); 3, only the large subunit was incubated as above (49 μg); 4, GGT sample which was not separated into subunits by HPLC. GGT activity staining was performed as described previously [9]. L, S, and Native indicate large subunit, small subunit, and GGT before separation into subunits, respectively.

the specific activity of the strain was approximately 3% of that of a strain harboring a plasmid encoding the intact structural gene. These results indicate that portions of the separated large and small subunits can be reconstituted in vitro, enzymatic activity thereby being exhibited, and that the separately expressed large and small subunits are able to associate in vivo and be folded into an active structure, although the specific activity of the associated subunits was much lower than that of the native enzyme [43].

6. Low temperature induction of GGT

As described previously, *E. coli* K-12 cells grown at 20°C showed high GGT activity, but only 14% of that at 37°C and little at 42°C [8]. It was also found that *E. coli* B and *Salmonella typhimurium* synthesized more GGT at 20°C than at 37°C, although the difference was not as great as in the case of *E. coli* K-12 [55]. The mechanism of low temperature induction of *E. coli* K-12 GGT was studied [55]. On Western

blot analysis, *E. coli* K-12 cells grown at 20°C produced a larger amount of the GGT protein than ones grown at 37°C. *E. coli* K-12 cells were cultured at 20, 37 and 42°C, and mRNA transcripts of the GGT gene (*ggt*) in the cells were quantified, respectively, by Northern blot analysis (Fig. 6). The level of *ggt* mRNA at 20°C was 10-fold higher than that at 37°C. These results show that the higher GGT activity of *E. coli* K-12 cells grown at 20°C is due mainly to the higher level of expression of the GGT protein at 20°C because of the higher level of *ggt* mRNA at 20°C. The transcription start point of the *E. coli* K-12 *ggt* gene was identified by means of the primer extension method and the promoter region was predicted by deletion analysis. Since the consensus sequence of the binding site for the major cold shock protein, CS7.4, was not found in the regulatory region of *ggt*, CS7.4 seems to have no relation with the low temperature induction of *E. coli* K-12 GGT. However, it was shown that the upstream region of *ggt* is critical for its low temperature induction. The physiological advantage of low temperature induction of GGT has not been elucidated yet.

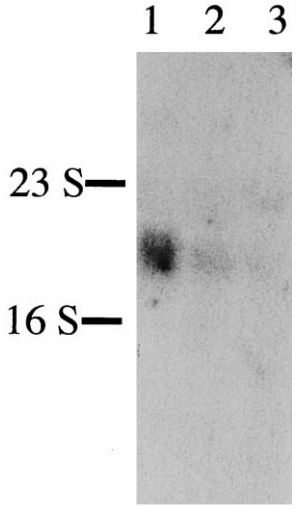

Fig. 6. Effect of temperature on the amount of *ggt* mRNA. Total RNA (30 μg) was isolated from wild-type *E. coli* K-12 cells grown at 20°C (lane 1), 37°C (lane 2), or 42°C (lane 3), and then separated by agarose gel electrophoresis, followed by Northern blot analysis using *ggt* as a DNA probe. 16S and 23S indicate the positions of 16S and 23S rRNA, respectively.

7. Crystallization and X-ray analysis of GGT

The crystallization of mammalian GGTs is difficult because they are heavily glycosylated with heterogeneous sugar chains. *E. coli* GGT is not glycosylated. Therefore, to study the structure–function relationship of GGT, a crystallographical study of *E. coli* GGT was performed. Since an overproducing strain of *E. coli* GGT was obtained by gene cloning and GGT can be purified readily [41], the conditions necessary to obtain appropriate GGT crystals for X-ray diffraction analysis were investigated [56]. Two kinds of crystals were obtained, one was isolated from ammonium sulfate and the other from PEG6000 as the precipitant. The crystals obtained with PEG6000 were relatively stable as for X-ray analysis and diffracted to 2.0 Å resolution. These crystals were used for further study. The crystals belonged to space group $P2_12_12_1$ with cell dimensions of $a = 128.1$, $b = 129.9$ and $c = 79.2$ Å. Brannigan et al. [47] compared the three-dimensional structures of three amidohydrolases whose crystal structures had been determined and which were known to be processed post-translationally. They found that all of them share an unusual fold in which the nucleophile and other catalytic groups occupy equivalent sites, and they named this structural superfamily of enzymes, the N-terminal nucleophile hydrolases. All of them have characteristic two antiparallel β-pleated sheets consisting of several β-strands. These enzymes use the side chain of the N-terminal amino acid residue of the B-chain as the nucleophile in the catalytic attack at the carbonyl carbon of substrates and this side chain was suggested to be responsible for their possible autoprocessing mechanism. Although X-ray analysis of *E. coli* GGT has not yet been completed, it has been shown that GGT also possesses the characteris-

tic two antiparallel β-pleated sheets [57]. This finding strongly suggests that the processing of GGT is autocatalytic and that the active center for GGT activity is Thr-391. We are in the process of completing the three-dimensional structure analysis of GGT and anticipate that it will be completed in the near future.

8. Industrial microbiological application of *E. coli* GGT

Since it is very troublesome to prepare periplasmic fractions, the industrial application of wild-type *E. coli* GGT is uneconomical. To overcome this problem, we used periplasmic leaky mutants and developed a rapid purification method for the enzyme [58]. Three excretory mutants, the *lky*, *tolA* and *tolB* strains, which pleiotropically release the periplasmic proteins of *E. coli*, were transformed with a plasmid containing the *ggt* gene. We found that a *tolA* strain excreted recombinant GGT into the medium very efficiently (about 70%). The excreted GGT could be easily purified to electrophoretic homogeneity with a relatively high yield by means of a simple two-step method consisting of ammonium sulfate fractionation and chromatofocusing.

Several γ-glutamyl peptides have been synthesized with γ-glutamylcysteine synthetase (EC 6.3.2.2) [59]. However, the drawbacks of utilizing this enzyme in industry are that an effective and economical ATP-supplying system is essential, and that a product, ADP, inhibits this enzyme strongly. Moreover, the substrate specificity of this enzyme is narrow. On the other hand, *E. coli* GGT does not require any energy source, such as ATP, and it can use many amino acids and peptides as γ-glutamyl acceptors and inexpensive L-glutamine as a good γ-glutamyl donor [9]. Therefore, we devised an enzymatic method for the synthesis of γ-glutamyl compounds with *E. coli* GGT. The γ-glutamylization of amino acids increases their solubility in an aqueous solution and protects the amino acids from degradative enzymes in organisms. Since γ-glutamyl residues are cleaved off from γ-glutamyl compounds to release amino acids in the organs where GGT exists, it is effective to use γ-glutamyl compounds to supply some specific compounds to organs such as kidneys rich in GGT activity. Wilk et al. [60] injected synthesized γ-glutamyl DOPA into mice and found that γ-glutamyl DOPA can be used as a kidney-specific dopamine precursor. We have developed enzymatic methods for the synthesis of γ-glutamyl-L-DOPA [61], γ-glutamyl-L-histidine [62], γ-glutamyl-L-tyrosine methyl ester [63], and *S*-benzyl–GSH monomethyl ester [64] in high yields (Table 2). Enzymatically synthesized γ-glutamyl-L-DOPA has been shown to be a possible pro-drug for Parkinson's disease [65], and to be a substrate for sensitive electrochemical measurement of the enzymatic activity of human serum GGT, which is a marker enzyme of hepatomas and alcoholic hepatic diseases [66]. γ-Glutamyl-L-cysteine was suggested to be useful for transfusion because a high concentration of cysteine in the serum is toxic for organisms [67], and γ-glutamyl dermorphin was also proved to be an effective

Table 2
Optimum conditions for the synthesis of γ-glutamyl amino acids by *E. coli* GGT

γ-Glutamyl amino acid	Concentration			pH	Yield	
	L-Gln (mM)	Acceptor (mM)	GGT (U/ml)		(%)	(g/l)
γ-Glu-L-DOPA	200	200	250	10.6	79	51.5
γ-Glu-L-His	300	300	200	9.7	48	41.2
γ-Glu-L-Tyr methyl ester	300	300	200	7.3	37	35.7
S-Benzyl–GSH monomethyl ester	200	100	200	6.2	76	31.2

pro-drug of an opioid peptide, dermorphin [68]. Therefore, the application of GGT isolated by the present procedure to the synthesis of γ-glutamyl compounds is promising.

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