JOURNAL OF BACTERIOLOGY, Sept. 1993, p. 6038–6040
0021-9193/93/186038-03$02.00/0

Vol. 175, No. 18

NOTES

Escherichia coli K-12 Can Utilize an Exogenous γ-Glutamyl Peptide as an Amino Acid Source, for which γ-Glutamyltranspeptidase Is Essential

HIDEYUKI SUZUKI,* WATARU HASHIMOTO, AND HIDEHIKO KUMAGAI

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan

Received 10 May 1993/Accepted 10 July 1993

***Escherichia coli* K-12 can utilize a γ-glutamyl peptide as an amino acid source, for which γ-glutamyltranspeptidase (EC 2.3.2.2) is essential. We suggest that the γ-glutamyl linkage of a γ-glutamyl peptide is hydrolyzed by γ-glutamyltranspeptidase located in the periplasmic space, and the released amino acid is taken up and utilized by *E. coli*.**

γ-Glutamyltranspeptidase (GGT) (EC 2.3.2.2) catalyzes the transfer of the γ-glutamyl moiety from γ-glutamyl compounds such as glutathione to amino acids and peptides and the hydrolysis of γ-glutamyl compounds (17). We have isolated GGT from the periplasmic space of *Escherichia coli* K-12 and examined its enzymatic characteristics (14). *E. coli* GGT can utilize various kinds of γ-glutamyl peptides as substrates for the hydrolysis reaction and as γ-glutamyl donors for the transpeptidation reaction. A GGT-deficient mutant of *E. coli* K-12 was isolated, and the gene coding for GGT (*ggt*) was mapped at 76 min on the *E. coli* chromosome (16). The only typical phenotype of the GGT-deficient mutant found was that the culture medium contained much more glutathione than that of the wild-type strain. The *ggt* gene has been cloned (12), and its nucleotide sequence has been determined (13). However, the physiological significance of GGT in *E. coli* is not known yet.

In this study, we constructed *E. coli* K-12 auxotrophs with a GGT-deficient mutation and investigated the role of GGT in utilization of an exogenous γ-glutamyl peptide as an amino acid source.

All γ-glutamyl peptides used were γ-L-glutamyl-L-amino acids and were purchased from Sigma Chemical Co. All strains used in this study were derivatives of *E. coli* K-12 and are listed in Table 1. *E. coli* K-12 derivatives were constructed by the P1 *vir* phage transduction according to the method of Miller (11). As a rich medium, LB broth (11) was used. M9 glucose plates (11) supplemented with thiamine (2 μg/ml) and appropriate amino acids (50 μg/ml) were used as minimal medium plates. The solid media contained 1.5% agar, and the soft agar contained 0.8% agar. To check the utilization of γ-glutamyl peptides, one scoop of each strain was picked up from the colonies grown on LB plates with a toothpick and suspended in 0.5 ml of saline. One microliter of the cell suspensions was spotted on the minimal medium plates supplemented with 50 to 100 μg of each peptide per ml, and the plates were incubated at 37°C.

Utilization of γ-glutamyl peptides as amino acids sources. Since GGT of *E. coli* catalyzes the hydrolysis of various γ-glutamyl compounds (14), we predicted that if we provided a histidine auxotroph with γ-glutamylhistidine exogenously, then GGT located in the periplasmic space would hydrolyze its γ-glutamyl linkage and the released histidine would be taken up and utilized by the cells. As expected, a histidine auxotroph (JP2144) grew on an M9 glucose plate supplemented with γ-glutamylhistidine, tryptophan, tyrosine, isoleucine, and valine but not on the same plate without γ-glutamylhistidine. A *ggt* derivative of JP2144 was obtained by P1 transduction, which was designated as SH761. This strain did not grow on the minimal medium plate supplemented with γ-glutamylhistidine as a histidine source, whereas the isogenic ggt^+ strain SH762 did grow on this plate (Table 2). Other combinations of γ-glutamyl peptides and auxotrophs were also examined. A phenylalanine auxotroph, tyrosine auxotroph, tryptophan auxotroph, and leucine auxotroph each grew on M9 glucose plates supplemented with γ-glutamylphenylalanine, γ-glutamyltyrosine, γ-glutamyltryptophan, and γ-glutamylleucine, respectively, as rapidly as they grew on the plate supplemented with each amino acid, whereas they did not grow on the same plates without each γ-glutamyl amino acid (Table 2). Moreover, *ggt* derivatives of each auxotroph did not grow on M9 glucose plates supplemented with each γ-glutamyl amino acid (Table 2). These results indicate that *E. coli* can utilize an exogenous γ-glutamyl peptide as an amino acid source and that GGT is essential for this.

One of the most abundant γ-glutamyl peptides existing in nature is glutathione, that is, γ-glutamylcysteinylglycine. Glutathione exists in many biological cells and protects the cells from reactive and toxic chemical compounds formed in the metabolism of oxygen and electrophilic xenobiotics (6). A cysteine auxotroph and a glycine auxotroph were examined to determine whether they could utilize glutathione as a cysteine source and a glycine source, respectively (Table 3). The cysteine auxotroph grew on an M9 glucose plate supplemented with glutathione as well as cysteine, cysteinylglycine, or γ-glutamylcysteine. However, a *ggt* derivative of the cysteine auxotroph did not grow on an M9 glucose plate supplemented with glutathione or γ-glutamylcysteine, but they grew on M9 glucose plates supplemented with cysteine

* Corresponding author.

TABLE 1. Strains used in this study

Strain	Genotype	Source and reference
C600 r^- m^-	F^- *thr-1 leuB6 thi-1 hsdS1 lacY1 tonA21 supE44* λ^-	B. J. Bachmann (CGSC5346)
JP2144	F^- *trpA9605*(Am) *tyrR366 his-85*(Am) *ilv-632 tsx-84* λ^-	B. J. Bachmann (CGSC5448) (1)
KA197	Hfr PO45 *thi-1 pheA97 relA1 spoT1* λ^-	B. J. Bachmann (CGSC5243)
ME8312	F^- *zfe-208*::Tn*10 glyA6* Δ(*argF-lac*)*U169 araD139 relA1 rpsL150 flb530 deoC1 tonA21 thi ptsF25*	A. Nishimura[a]
MG1655	F^- prototrophic	C. A. Gross
N3087	F^- *tyrA16*::Tn*10* IN(*rrnD-rrnE*)*1* λ^-	B. J. Bachmann (CGSC6662)
SH162	F^- *trpA9761* Δ*trpDC401* IN(*rrnD-rrnE*)*1* λ^-	C. Yanofsky (5)
SH639	F^- *ggt-2*	Laboratory stock
SH682	F^- *ggt-2 zhg*::Tn*10*	16
SH690	Hfr PO1 *thi-1 cysG44 relA1 spoT1* λ	Laboratory stock
SH691	Hfr PO1 *thi-1 cysG44 relA1 spoT1 ggt-1* λ^-	Laboratory stock
SH761	F^- *his-85*(Am) *ggt-2 zhg*::Tn*10 tsx-84 trpA9605*(Am) *tyrR366 ilv-632* λ^-	JP2144 × P1(SH682), this work
SH762	F^- *his-85*(Am) *zhg*::Tn*10 tsx-84 trpA9605*(Am) *tyrR366 ilv-632* λ^-	JP2144 × P1(SH682), this work
SH772	Hfr PO45 *pheA97 zhg*::Tn*10 relA1 spoT1* λ^-	KA197 × P1(SH682), this work
SH773	Hfr PO45 *pheA97 ggt-2 zhg*::Tn*10 relA1 spoT1* λ^-	KA197 × P1(SH682), this work
SH774	F^- *tyrA16*::Tn*10*	MG1655 × P1(N3087), this work
SH775	F^- *tyrA16*::Tn*10 ggt-2*	SH639 × P1(N3087), this work
SH786	Hfr PO1 *cysG44 thi-1 relA1 spoT1 zfe-208*::Tn*10* λ^-	SH690 × P1(ME8312), this work
SH788	Hfr PO1 *cysG44 ggt-1 thi-1 relA1 spoT1 zfe-208*::Tn*10* λ^-	SH691 × P1(ME8312), this work
SH792	F^- *trpA9761* Δ*trpDC401 ggt-2 zhg*::Tn*10*	SH162 × P1(SH682), this work
SH793	F^- *trpA9761* Δ*trpDC401 zhg*::Tn*10*	SH162 × P1(SH682), this work
SH794	F^- *leuB6 ggt-2 zhg*::Tn*10 thr-1 thi-1 hsdS1 lacY1 tonA21 supE44* λ^-	C600 r^- m^- × P1(SH682), this work
SH795	F^- *leuB6 zhg*::Tn*10 thr-1 thi-1 hsdS1 lacY1 tonA21 supE44* λ^-	C600 r^- m^- × P1(SH682), this work
SH802	F^- *glyA6 zfe-208*::Tn*10*	MG1655 × P1(ME8312), this work
SH804	F^- *glyA6 ggt-2 zfe-208*::Tn*10*	SH639 × P1(ME8312), this work

[a] National Institute of Genetics, Mishima, Japan.

or cysteinylglycine. Similar results were obtained with the glycine auxotroph. The glycine auxotroph grew on an M9 glucose plate supplemented with glutathione as well as glycine, cysteinylglycine, or γ-glutamylglycine. However, a *ggt* derivative of the glycine auxotroph did not grow on an M9 glucose plate supplemented with glutathione or γ-glutamylglycine, but it grew on an M9 glucose plate supplemented with glycine or cysteinylglycine. These results indicate that *E. coli* can utilize exogenous glutathione as a cysteine source and a glycine source, as well as γ-glutamylcysteine and γ-glutamylglycine, and that GGT is essential for this. Oxidized glutathione was also utilized as a cysteine source and a glycine source (data not shown). One of the important physiological roles of GGT in *E. coli* could be a catalysis of the initial step of salvage or recycle of cysteine into the cell as was reported by other researchers (3, 4).

When the GGT activity of intact cells was measured, the transpeptidation activity was found to be negligible. Besides, since the K_m values for γ-glutamyl acceptors were extremely high (14), it is hard to imagine that the transpeptidation reaction has any physiological significance in *E. coli*. Therefore, we suggest that the γ-glutamyl linkage of a γ-glutamyl amino acid is hydrolyzed by GGT located in the periplasmic space and that the released amino acid is taken up and utilized by *E. coli*. It has been reported that dipeptidases D, E, and Q and aminopeptidases A, B, N, and P of *Salmonella typhimurium* and *E. coli* hydrolyze peptides supplied exogenously and allow the peptides to be used as amino acid sources (2, 7–10). However, GGT-deficient mutants cannot utilize γ-glutamyl peptides as amino acid sources (Tables 2 and 3); and, vice versa, peptides whose N terminals are masked by a γ-glutamyl linkage are not cleaved by these dipeptidases and aminopeptidases in cells. Miller and his coworkers reported that peptidases are involved in the

TABLE 2. Utilization of γ-glutamyl amino acids

Strain	Genotype	Growth on M9 glucose medium alone or supplemented with[a]:										
		Alone	His	γ-Glu-His	Phe	γ-Glu-Phe	Tyr	γ-Glu-Tyr	Trp	γ-Glu-Trp	Leu	γ-Glu-Leu
MG1655	Wild type	+	+	+	+	+	+	+	+	+	+	+
SH761	*ggt his*	−	+	−								
SH762	*his*	−	+	+								
SH773	*ggt phe*	−			+	−						
SH772	*phe*	−			+	+						
SH775	*ggt tyr*	−					+	−				
SH774	*tyr*	−					+	+				
SH792	*ggt trp*	−							+	−		
SH793	*trp*	−							+	+		
SH794	*ggt leu*	−									+	−
SH795	*leu*	−									+	+

[a] Plates were incubated overnight at 37°C. γ-Glu, γ-glutamyl.

TABLE 3. Utilization of glutathione and related compounds by cysteine and glycine auxotrophs

Strain	Genotype	Growth on plates containing[a]:							
		M9 glucose medium alone or supplemented with:							LB
		Alone	Cys	Gly	γ-Glu-Cys	γ-Glu-Gly	Cys-Gly	GSH	
SH786	*cysG*	−	+	ND[b]	+	ND	+	+	+
SH788	*cysG ggt-1*	−	+	ND	−	ND	+	−	+
SH802	*glyA6*	−	ND	+	ND	+	IC[c]	IC	+
SH804	*glyA6 ggt-2*	−	ND	+	ND	−	IC	−	+

[a] Plates were incubated two overnight at 37°C. γ-Glu-Cys, γ-glutamylcysteine; γ-Glu-Gly, γ-glutamylglycine; Cys-Gly, cysteinylglycine; GSH, glutathione.
[b] ND, not determined.
[c] IC, inconfluent growth.

degradation of intracellular proteins (7, 8, 19, 20). However, a peptide which contains a γ-glutamyl linkage, such as peptidoglycan, would not be cleaved completely without GGT. Since GGT can hydrolyze γ-D-glutamyl peptides as well as γ-L-glutamyl peptides (14), and since it is located in the periplasmic space (15), in which the peptidoglycan is located, GGT may play some role in hydrolyzing the γ-glutamyl linkage in the peptidoglycan.

Selective phenotype of a GGT-deficient mutant on a γ-glutamylvaline plate. Since the selective phenotype of a GGT-deficient mutant was not known, in a previous study (16) we isolated GGT-deficient mutants by means of screening the GGT activity of mutagenized cells, using a colorimetric method involving γ-glutamyl-*p*-nitroanilide. *E. coli* K-12 is valine sensitive and cannot grow on a minimal medium plate supplemented with valine without isoleucine. This is because two acetohydroxy acid synthases of *E. coli* K-12, which catalyze the synthesis of valine and isoleucine, are strongly inhibited by the end product, valine (18). Since we found that *E. coli* K-12 can utilize a γ-glutamyl peptide as an amino acid source and that GGT is essential for this, as described above, γ-glutamylvaline was used in an attempt to select a GGT-deficient mutant. Aliquots (10 μl) of overnight cultures of MG1655 and SH639 were mixed with 3 ml of M9 soft agar, respectively, and then spread on M9 glucose plates. Then, small amounts of γ-glutamylvaline, valine, and a mixture of valine and isoleucine were spotted on the plates, and the plates were incubated at 37°C (data not shown). The wild-type strain (MG1655) grew where the mixture of valine and isoleucine had been spotted but not where γ-glutamylvaline or valine had been spotted. A GGT-deficient strain (SH639), however, could also grow where γ-glutamylvaline had been spotted. This indicates that γ-glutamylvaline itself is not toxic for *E. coli*; but when valine is released from it by GGT, the valine prevents the growth of *E. coli*. Growth on a γ-glutamylvaline plate is a selective phenotype of a GGT-deficient mutant.

This work was supported by Research Grants-in-Aid for Scientific Research, no. 02304035 and 04856024, from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. **Camakaris, H., and J. Pittard.** 1973. Regulation of tyrosine and phenylalanine biosynthesis in *Escherichia coli* K-12: properties of the *tyrR* gene product. J. Bacteriol. **115:**1135–1144.
2. **Carter, T. H., and C. G. Miller.** 1984. Aspartate-specific peptidases in *Salmonella typhimurium*: mutants deficient in peptidase E. J. Bacteriol. **159:**453–459.
3. **Cho, E. S., N. Johnson, and B. C. F. Snider.** 1984. Tissue glutathione as a cyst(e)ine reservoir during cystine depletion in growing rats. J. Nutr. **114:**1853–1862.
4. **Higashi, T., N. Tateishi, A. Naruse, and Y. Sakamoto.** 1977. A novel physiological role of liver glutathione as a reservoir of L-cystein. J. Biochem. **82:**117–124.
5. **Jackson, E. N., and C. Yanofsky.** 1972. Internal deletions in tryptophan operon of *Escherichia coli*. J. Mol. Biol. **71:**149–161.
6. **Meister, A., and M. E. Anderson.** 1983. Glutathione. Annu. Rev. Biochem. **52:**711–760.
7. **Miller, C. G.** 1985. Genetics and physiological roles of *Salmonella typhimurium* peptidases, p. 346–349. *In* L. Leive, P. F. Bonventre, J. A. Morello, S. Schlesinger, S. D. Silver, and H. C. Wu (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
8. **Miller, C. G.** 1985. Protein degradation and proteolytic modification, p. 680–691. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
9. **Miller, C. G., and K. MacKinnon.** 1974. Peptidase mutants of *Salmonella typhimurium*. J. Bacteriol. **120:**355–363.
10. **Miller, C. G., and G. Schwartz.** 1978. Peptidase-deficient mutants of *Escherichia coli*. J. Bacteriol. **135:**603–611.
11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Suzuki, H., H. Kumagai, T. Echigo, and T. Tochikura.** 1988. Molecular cloning of *Escherichia coli* K-12 *ggt* and rapid isolation of γ-glutamyltranspeptidase. Biochem. Biophys. Res. Commun. **150:**33–38.
13. **Suzuki, H., H. Kumagai, T. Echigo, and T. Tochikura.** 1989. DNA sequence of the *Escherichia coli* K-12 γ-glutamyltranspeptidase gene, *ggt*. J. Bacteriol. **171:**5169–5172.
14. **Suzuki, H., H. Kumagai, and T. Tochikura.** 1986. γ-Glutamyltranspeptidase from *Escherichia coli* K-12: purification and properties. J. Bacteriol. **168:**1325–1331.
15. **Suzuki, H., H. Kumagai, and T. Tochikura.** 1986. γ-Glutamyltranspeptidase from *Escherichia coli* K-12: formation and localization. J. Bacteriol. **168:**1332–1335.
16. **Suzuki, H., H. Kumagai, and T. Tochikura.** 1987. Isolation, genetic mapping, and characterization of *Escherichia coli* K-12 mutants lacking γ-glutamyltranspeptidase. J. Bacteriol. **169:** 3926–3931.
17. **Tate, S. S., and A. Meister.** 1981. γ-Glutamyl transpeptidase: catalytic, structural and functional aspects. Mol. Cell. Biochem. **39:**357–368.
18. **Umbarger, H. E.** 1983. The biosynthesis of isoleucine and valine and its regulation, p. 245–266. *In* K. M. Herrmann and R. L. Somerville (ed.), Amino acids: biosynthesis and genetic regulation. Addison-Wesley Publishing, Reading, Mass.
19. **Yen, C., L. Green, and C. G. Miller.** 1980. Degradation of intracellular protein in *Salmonella typhimurium* peptidase mutants. J. Mol. Biol. **143:**21–33.
20. **Yen, C., L. Green, and C. G. Miller.** 1980. Peptide accumulation during growth of peptidase deficient mutants. J. Mol. Biol. **143:**35–48.