Kor. J. Appl. Microbiol. Biotech.
Vol. 18, No. 1, 103-108 (1990)

총설 (**Review**)

Enzymatic and Genetic Aspects of Glyoxalase I in Microorganisms

Rhee, Hae-Ik

Department of Applied Biotechnology, Kangweon National University, Chuncheon 200-701, Korea

미생물에 있어서 글리옥살라아제 I의 효소학적, 유전학적 고찰

이 해 익

강원대학교 생물응용공학과

The enzymatic studies on the methylglyoxal metabolism in yeast and bacterial cells indicated that organisms are equipped with the common and manifold systems for the detoxification of methylglyoxal. Among these systems, the glyoxalase I is the most important route for methylglyoxal detoxification. The molecular structure of glyoxalase I is apparently distinct from the enzyme sources, and zinc ion is an essential cofactor in enzyme activity. The gene for *Pseudomonas putida* glyoxalase I functioned as a scavenger of methylglyoxal and regulated the cell size of the bacterium. Comparison of the nucleotide sequence of the *P. putida* glyoxalase I gene with the N-terminal amino acid sequence of the purified enzyme revealed that the N-terminal methionine residue was removed after translation. Possible physiological role of glyoxalase I was also discussed.

Methylglyoxal is a highly toxic compound being able to arrest the growth of cells from microorganisms to mammals at the millimolar concentrations (1-5). Since the excellent work of Szent-Gyorgyi and his coworkers, 2-oxoaldehydes, such as methylglyoxal, have been receiving increased interest for their role in the regulation of cell proliferation (6).

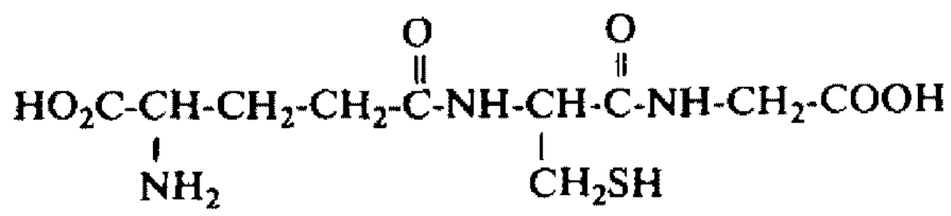
Methylglyoxal is metabolized by various routes. The glyoxalase system, consisting of glyoxalase I and glyoxalase II, has been considered to have a ubiquitous in the detoxification of 2-oxoaldehydes (7, 8). In addition to the glyoxalase system, a reduction/oxidation systems has been recently reported to be functioning in the detoxification of 2-oxoaldehydes in cells (9-12). In this system, methylglyoxal is converted to lactate via lactaldehyde by the sequential actions of methylglyoxal reductase and lactaladehyde dehydrogenase. A direct oxidation route of methylglyoxal to pyruvate has been known to exist in goat liver and the bacterium *Pseudomonas putida* and the reaction is catalyzed by NAD(P)-linked methylglyoxal dehydrogenase (13, 14).

The regulation of synthesis and degradation of methylglyoxal in mammalian, plant and microbial cells is of increased interest especially from the standpoint of the regulation of cell division. The enzymatic and genetic studies on the glyoxalase I indicated that glyoxalase system is functioning not only as a detoxification of methylglyoxal, but also as a regulation of microbial cell proliferation. In this article, the enzymatic and genetic approaches of glyoxalase I and its some physiological roles are described.

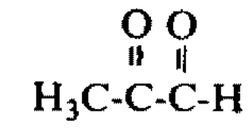
Key words: Glyoxalase I, Methylglyoxal, Cell proliferation, Detoxification.
*Corresponding author

Enzymatic Characteristics of Glyoxalase I

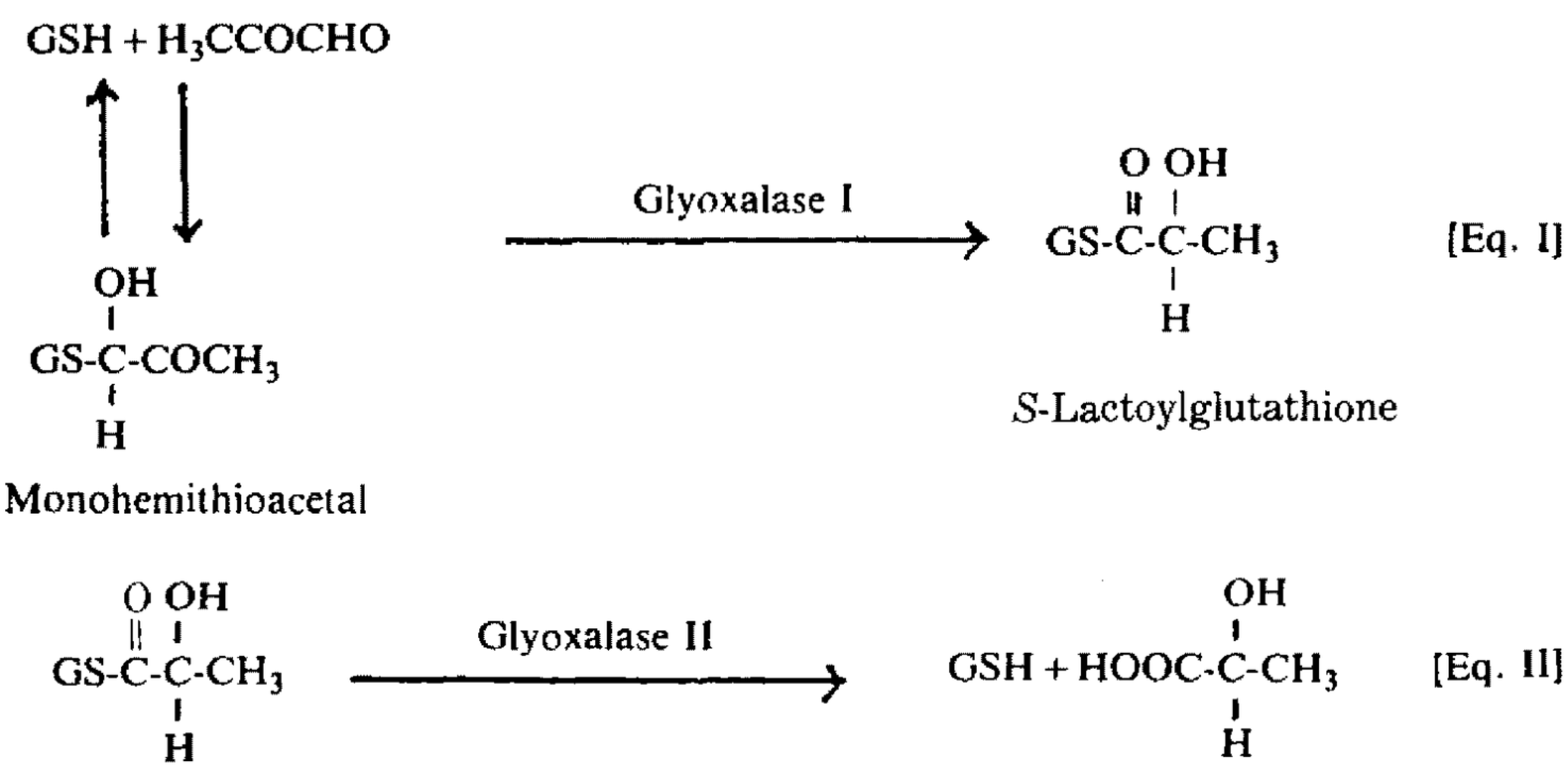
The glyoxalase system was discovered in 1913 and



Glutathione (GSH)



Methylglyoxal



[Eq. I]

[Eq. II]

consists of two enzymes and their cofactor, glutathione. Glyoxalase I (*S*-lactoylglutathione lyase, EC 4.4.1.5) converts the monohemithioacetal formed from glutathione and methylglyoxal or other 2-oxoaldehydes into *S*-lactoylglutathione as shown in Equation I. This thioester is then hydrolyzed by glyoxalase II (hydroxylacylglutathione hydrolase, EC 3.1.2.6), as shown in Equation II, to produce lactic acid and free glutathione.

Glyoxalase I is widely distributed and has been purified and characterized from rat liver (15), pig (16), human erythrocytes (17), sheep liver (18), yeast *Saccharomyces cerevisiae* (19, 20), mold *Aspergillus niger* (21) and from bacterium *Pseudomonas putida* (22). The molecular structure of glyoxalse I is apparently distinct from the enzyme sources. The molecular weight of mammalian glyoxalase I is approxiamtly 46,000-48,000 and consists of two identical subunits with one zinc atom per subunit. On the other hand, the molecular weight of prokaryotic and eukaryotic microorganisms is in a range 20,000-36,000 and consists of a single polypeptide chain with one zinc atom per enzyme. Zinc ion is an essential cofactor in glyoxalase I activity and the activity of the enzyme is lost by treating the enzymes with chelators. However, metal free apoenzyme can be effiencetly reactivated by the addition of other metal ions such as Mg^{+2} and Ca^{+2} (16, 18, 20, 23). Further study is needed on the function of metal ions in glyoxalase I reaction. Recently, the yeast glyoxalse I has been shown to contain 0.75% (w/w) carbohydrate consisting of fucose, mannose, galactose, manosamine, *N*-acetylneuraminate and possibly glucose (24).

The glyoxalase I react with hemithioacetal, a non-enzymatic condensation product between glutathione and 2-oxoaldehydes such as methylglyoxal, phenylglyoxal and 4,5-dioxovalerate, as shown in Eq. I. The utilization of 4,5-dioxovalerate, a precursor for 5-aminolevulinate, by glyoxalase I is of physiological significance in the regulation of porphyrin synthesis (25-27). The strong inhibition of glyoxalase I activity by porphyrin compounds may support this possibility (28). However closer inspection should be paid on this hypothesis, because 4,5-dioxovalerate is also utilized by methylglyoxal metabolizing enzymes (12, 14).

Genetic and Physiological Characters of Glyoxalase I

The gene for the *Pseudomonas putida* glyoxalase I has been cloned as a gene that increases resistance of *Escherichia coli* cells to methylglyoxal and the resultant hybrid plasmid was designated pGI423 (29). The introduction of pGI423 into *E. coli* cells with resulted in a marked increase in glyoxalase I activity (150 fold). The *E. coli* cells transformed with pGI423 can grow normally in the presence of 1.0 mM methylglyoxal, although *E. coli* cells with pBR322

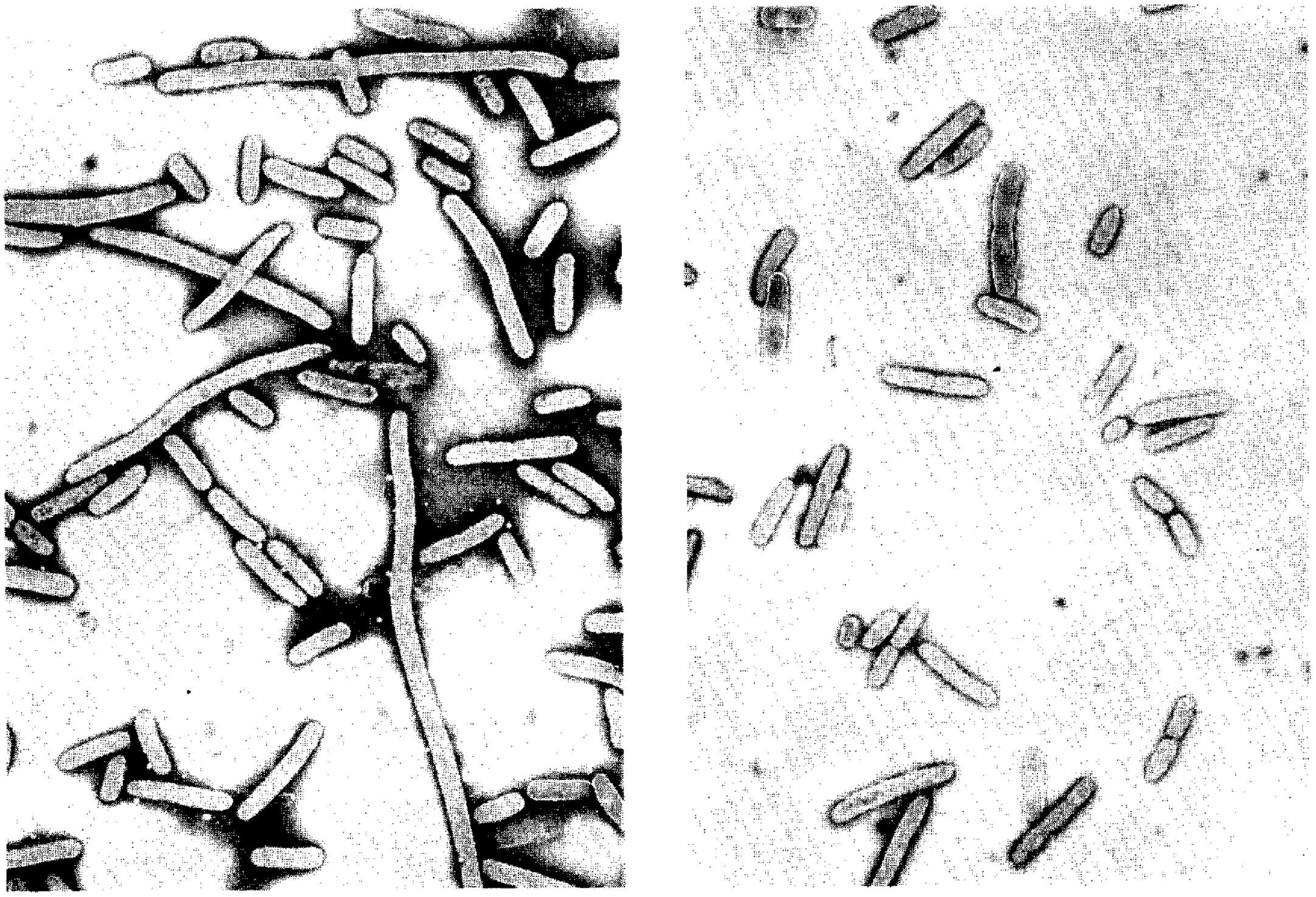


Fig. 1. Effect of glyoxalase I gene on bacterial cells size (29).
The bacterial cells of *Escherichia coli* C600 with pBR 322 (left) and *E. coli* C600 with pGI423 (right) were cultured to log phase in Davis-Mingioli minimal medium and methylglyoxal was added at 1.0 mM to cultures and the incubation was continued for 24 h.

shows abnormally stretched cell shape in a medium containing 1.0 mM methylglyoxal (Fig. 1). The result indicates that methylglyoxal itself has a capability to arrest the cell growth and converts the cell shape or size, possibly by inhibiting the DNA replication. The nucleotide sequence of the *P. putida* glyoxalase I gene was determined and amino acid sequence of the enzyme was deduced (Fig. 2) (30). The molecular weight of the enzyme deduced from the nucleotide sequence is 18,442, closely similar to that estimated with purified enzyme (molecular weight, 20,000) (22).

The N-terminal amino acid determined with the purified enzyme is serine, whereas the the nucleotide sequence of the gene directed methionine, thus suggesting the post-translational processing of N-terminal methionine of the enzyme. This may explain the localization of the glyoxalase I system in periplasmic space, though some arguments are raised as to the sitution of the system.

To know the function of the pathway, the change in activities of glyoxalase I and methylglyoxal reductase have been studied in relation to the yeast cell growth. The yeast glyoxalase I activity is remarkably affected by the nutritional conditions and 15-30 fold increase in the enzyme activity was observed, when the yeast cells are grown in a medium containing glycerol as a sole source of carbon (31) or L-threonine as a nitrogen source (32). This increase in glyoxalase I activity is presumably due to the detoxification of methylglyoxal formed through the catabolism of glycerol or L-threonine.

The inability of glyoxalase I deficient yeast mutants to grow these media supports above consideration (31, 33). Methylglyoxal reductase and other enzyme activities in the glycolytic bypath were not affected by the nutritional conditions. Thus, the results indicated the ubiquitous function of glyoxalase system, especially glyoxalase I, in the detoxification of methylglyoxal.

The study on yeast *Saccharomyces cerevisiae*

TTTTTTCACAGCTTGTCGGCCATGATCAATTCAGCTAACGGATTAGCGACCCCATCTGCGCACACGC

GGGCCTCTAATTTCGCACGTCGCCCTCGTGCAGGCGCTTTCATTTGCCTCCGCGCTCACCTCCCGT
-35

ATCATGGACGGCCTGTCTTACCGCTGGCGCCTGCGGGCCCGGCCTCGTCTCTGAGCTTC ATG AGC
-10 Met Ser

CTG AAC GAC CTC AAC ACC CTG CCT GCG TTA CTG CCC AAG CGG ACC CTG CCA
Leu Asn Asp Leu Asn Thr Leu Pro Ala Leu Leu Pro Lys Arg Thr Leu Pro

CTG CGC AAT TCG TTT CAA CCA CAC CAT CGT GCG CGT CAA GGA CAT CGA GAA
Leu Arg Asn Ser Phe Gln Pro His His Arg Ala Arg Gln Gly His Arg Glu

GTC GCT GAC TCT ATA CCC GCG TGC TTG GTT TCA AAC TGG TGG ACA AGC GAC
Val Ala Asp Ser Ile Pro Ala Cys Leu Val Ser Asn Trp Trp Thr Ser Asp

TTC GTC GAA GCC AAG TTC AGC CTG TAC TTC CTG GCG CTG GTT GAC CCA GCG
Phe Val Glu Ala Lys Phe Ser Leu Tyr Phe Leu Ala Leu Val Asp Pro Ala

ACA ATT CCA GCT GAT GAC GAT GCG CGT CAC CAG TGG ATG AAG TCG ATC CCT
Thr Ile Pro Ala Asp Asp Asp Ala Arg His Gln Trp Met Lys Ser Ile Pro

GGC GTA CTT GAG TTG ACG CAT AAC CAC GGC ACT GAA CGT GAT GCA GAC TTC
Gly Val Leu Glu Leu Thr His Asn His Gly Thr Glu Arg Asp Ala Asp Phe

GCC TAT CAC GAT GGC AAT ACC GAC CCG CGC GGT TTT GGC CAT ATC TGC GTT
Ala Tyr His Asp Gly Asn Thr Asp Pro Arg Gly Phe Gly His Ile Cys Val

TCG GTG CCT GAC GTA GTT GCG GCG TGC GAA CGC TTC GAG GCC TTG CAG GTG
Ser Val Pro Asp Val Val Ala Ala Cys Glu Arg Phe Glu Ala Leu Gln Val

CCG TTC CAG AAG CGA CTG AGC GAC GGC CGT ATG AAC CAT CTG GCC TTT ATC
Pro Phe Gln Lys Arg Leu Ser Asp Gly Arg Met Asn His Leu Ala Phe Ile

AAA GAC CCG ATG GTT ACT GGG TCG AAG TGA TTCAGCCAACGCCCTTGTAAGTACAGC
Lys Asp Pro Met Val Thr Gly Ser Lys ***

TAACTAGCGCCCCGCGCATAGGCAACTGCAGTGGGGCGCACGCGGTATACAGCACAAACTGCTCCAA
IR IR
CGCTTGCAGGGCAATCACCTCAAGCCCTGTGATGACCGGTTTGCCCAGTGCTTCGGCGCGGCAGATC

AGTGGTGTGCGCGCGGCATCGCCACGACGTCGAAGACCGCTCTGCCATGCAATCGCGGTTTCGGGAA

Fig. 2. Nucleotide sequence of *Pseudomonas putida* glyoxalase I gene (30).
The amino and sequence of the glyoxalase I predicted from the nucleotide sequence. Several regulatory sequences flanking the glyoxalase I gene are indicated with underlines.

CDC mutants indicated that the glyoxalase I activity is raised when the yeast cells are allowed to grow and the activity being lowered in G_1-arrested cells (33, 34), thus indicating that glyoxalase I activity is related to cell growth regulation. Recently, Murata *et al.* reported a certain protein being capable of inactivating the glyoxalase I (antizyme) (33).

The regulation of the *P. putida* glyoxalase system has not been studied extensively, yet.

Conclusion and Perspective

Enzymatic and genetic studies on the methylglyoxal metabolism in yeast and bacterial cells indicated that organisms are equipped with the common and manifold systems for the detoxification of methylglyoxal. Among these systems, the glyoxalase I is the most important route for methylglyoxal detoxification. As for the biological siginificance of

methylglyoxal, Szent-Gyorgyi *et al.* have suggested the possiblity that methylglyoxal has a role in the regulation of cell division (35-37). Recent findings on the glyoxalase I status have indicated a direct relationship between glyoxalase I activity and proliferative states of cells, and the unregulated growth of cancer cells should be partly ascribed to the inactivation of 2-oxoaldehydes by the glyoxalase system before they reach their target in the machinery of cell division (3, 34). On the other hand, Gillespie (38, 39) presented the hypothesis that level of S-lactoylglutathione, a product of glyoxalase I reaction, and his provided an evidence that an increased level of *S*-lactoylglutathione is related to the tumor-promoting properties of the compound. The regulation of glyoxalase I is operating to accelerate the accumulation of *S*-lactoylglutathione. The growth inhibitory effect of 2-oxoaldehydes and the growth-promoting effect of *S*-lactoylglutathione are the reason why the inhibitors of glyoxalase I have been thought to be promising anticancer chemicals. Carcinostatic activities of various glyoxal derivatives toward certain tumors and leukemia support this possibility (1). Recent survey on the function of *S*-lactoylglutathione revealed the potent inflammatory effect of the glutathione thiolester (personal communication from Murata K.). The enzymatic production of *S*-lactoylglutathione has just recently been developed by applying glyoxalase I to glycerol-grown cells of *S. cerevisiae* and *E. coli* cells dosed with *P. putida* glyoxalase I gene (40, 41).

Besides of cell proliferation, a number of proposals concerning the natural role of glyoxalase I have been made. It has been suggested that it plays a role in the enhancement of anti-(immunoglobulin E)-induced histamine release (38), in microtuble assembly (42), in protection against intestinal bacteria (16), in postulated metabolic cycle for the degradation of glycine and threonine (32), and is possibly involved in the heme biosynthetic pathway (25-27). In spite of all this, little is known about the basic biological role of the glyoxalase system. Closer inspections on glyoxalase I status is now required to arrive at any definite conclusion on the function of the glyoxalase system.

요 약

효모나 세균을 이용한 메칠글리옥살 대사의 효소학적, 유전학적 연구로부터 생물체들은 메칠글리옥살의 해독을 위하여 보편적이고 다양한 경로를 가지고 있음을 알았다. 이들 대사경로 가운데 글리옥살라아제 I은 메칠글리옥살 해독에 있어서 가장 중요한 경로이다. 글리옥살라아제 I의 분자구조는 효소의 기원에 따라 크게 다르게 나타났고, 아연 이온은 효소활성에 필수적이었다. *Pseudomonas putida*의 글리옥살라아제 I은 유전자는 메칠글리옥살의 제거제로 작용하였고, 또한 세균의 크기를 조절하는 역할을 가지고 있었다. 본 유전자의 염기배열과 정제효소의 아미노 말단을 비교해 본 결과 아미노 말단의 메치오닌 잔기는 번역 후 제거됨을 알았다. 그밖에 글리옥살라아제 I의 생리적 역할에 대해서도 논의하였다.

References

1. Szent-Gyorgyi, A. and J.A. McLaughlin: *Science.*, **140**, 1391 (1963).
2. Schemeer, M.R.: *Science.*, **144**, 413 (1964).
3. Szent-Gyorgyi, A., L.G. Egyud and J.A. McLaughlin: *Science.*, **155**, 539 (1967).
4. Egyud, L.G. and A. Szent-Gyorgyi: *Proc. Natl. Acad. Sci. USA.* **55**, 388 (1966).
5. Murata, K., T. Saikusa, K. Watanabe, Y. Fukuda, M. Shimosaka, Y. Inoue and A. Kimura: *Eur. J. Biochem.*, **157**, 297 (1986).
6. Szent-Gyorgyi, A. and A. Hegyeli: *Biol. Bull.*, **123**, 466 (1962).
7. Dakin, H.D. and H.W. Dudley: *J. Biol. Chem.*, **14**, 155 (1913).
8. Neuberg, C.: *Biochem. Z.*, **49**, 502 (1914).
9. Monder, C.: *J. Biol. Chem.*, **242**, 4603 (1957).
10. Ray, M. and S. Ray: *Biochim. Biophys. Acta.*, **802**, 119 (1984).
11. Murata, K., Y. Fukuda., M. Shimosaka, K. Wastanabe, T. Saikusa and A. Kimura: *Eur. J. Biochem.*, **151**, 631 (1985).
12. Inoue, Y., H.-I, Rhee, K. Watanabe, K. Murata, A. Kimura: *Eur. J. Biochem.*, **171**, 213 (1988).
13. Ray, M. and S. Ray: *J. Biol. Chem.*, **257**, 10566 (1982).
14. Rhee, H.-I., K. Watanabe, K. Murata and A. Kimura: *Agric. Biol. Chem.*, **51**, 1059 (1987).
15. Marmstal, E. and B. Mannervik: *Biochim. Biophys. Acta.*, **566**, 362 (1979).
16. Aronsson, A.-C., and B. Mannervik: *Biochem. J.*, **165**, 503 (1977).
17. Aronsson, A.-C., G. Tibbelin and B. Mannervik: *Anal. Biochem.*, **92**, 390 (1979).
18. Uotila, L. and M. Koivusalo: *Eur. J. Biochem.*, **52**, 493 (1975).

19. Marmstal, E., A.-C, Aronsson and B. Mannervik: *Biochem. J.*, **183**, 23 (1979).
20. Murata, K., T. Saikusa, K. Watanabe, Y. Inoue, Y. Fukuda, M. Shimosaka and A. Kimura: *Agric. Biol. Chem.*, **50**, 1381 (1986).
21. Inoue, Y., H.-I. Rhee, K. Watanabe, K. Murata and A. Kimura: *J. Biochem.*, **102**, 583 (1987).
22. Rhee, H.-I., K. Murata and A. Kimura: *Biochem. Biophys. Res. Commun.*, **141**, 993 (1986).
23. Mannervik, B., L. Lindsstrom and T. Bartfai: *Eur. J. Biochem.*, **29**, 276 (1972).
24. Douglas, K.T., A.P. Seddon and Y. Nakagawa: *Int. J. Biochem.*, **18**, 549 (1986).
25. Jerzykowski, T., R. Winter and W. Matuszewski: *Biochem. J.*, **135**, 713 (1973).
26. Stafforini, D.M., J.P. Kushner and B.F. Burnham: *Biochim. Biophys. Acta.*, **839**, 191 (1985).
27. Rhee, H.-I., K. Murata and A. Kimura: *J. Biochem.*, **103**, 1045 (1988).
28. Douglas, K.T., I.N. Nadbi G.-S Sgarif: *IRCS Med. Sci.*, **10**, 86: (1982).
29. Rhee, H.-I., K. Murata and A. Kimura: *Biochem. Biophys. Res. Commun.*, **147**, 831 (1987).
30. Rhee, H.-I., N. Sato, K. Murata and A. Kimura: *Agric. Biol. Chem.*, **52**, 2243 (1988).
31. Penninckx, M.J., C.J. Jaspers and M.J. Legrain: *J. Biol. Chem.*, **258**, 6030 (1983).
32. Murata, K., T. Saikusa, K. Watanaabe, Y. Fukuda, M. Shimosaka, Y. Inoue and A. Kimura: *Eur. J. Biochem.*, **157**, 297 (1986).
33. Murata, K. and A. Kimura: *Protein, Nucleic acid, Enzyme.*, **31**, 1010 (1986).
34. Dudani, A.K., L.K. Srivastava and R. Prasad: *Biochem. Biophys. Res. Commun.*, **119**, 362 (1984).
35. Szent-Gyorgyi, A.: *Science.*, **149**, 34 (1965).
36. Egyud, L. G. and A. Szent-Gyorgyi: *Proc. Natl. Acad. Sci. USA.*, **56**, 203 (1966).
37. Fordor, G., J.P. Sachetto, A. Szent-gyorgyi and L.G. Egyud: *Proc. Natl. Acad. Sci. USA.*, **57**, 1644 (1967).
38. Gillespie, E.: *Nature.*, **277**, 135 (1979).
39. Gillespie, E.: *Biochem. Biophys. Res. Commun.*, **98**, 463 (1981).
40. Rhee, H.-I. and A. Kimura: *Protein, Nucleic acid, Enzyme.*, **33**, 1610 (1988).
41. Kosugi, N., Y. Inoue, H.-I. Rhee, K. Murata and A. Kimura: *Appl. Microbiol. Biotechnol.*, **28**, 263 (1988).
42. Gillespie, E.: *Fed. Proc.*, **34**, 541 (1975).

(Received August 18, 1989)