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Regulatory Properties of Glutamate Dehydrogenase from *Sulfolobus solfataricus*

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The purified glutamate dehydrogenase (GDH) from *Sulfolobus solfataricus* showed remarkable thermostability and retained 90–95% of the initial activity after incubation at −20°C, 4°C , and 25°C for up to 6 months. Unlike mammalian GDHs, the activity of GDH from *Sulfolobus solfataricus* was not significantly affected by the presence of various allosteric effectors such as ADP, GTP, and leucine. Incubation of GDH with increasing concentration of o-phthalaldehyde resulted in a progressive decrease in enzyme activity, suggesting that the o-phthalaldehyde-modified lysine or cysteine is directly involved in catalysis. The inhibition was competitive with respect to both 2-oxoglutarate (K_i = 30 μM) and NADH (K_i = 100 μM), further supporting a possibility that the o-phthalaldehyde-modified residues may be directly involved at the catalytic site. The modification of GDH by the arginine-specific dicarbonyl reagent phenylglyoxal was also examined with the view that arginine residues might play a general role in the binding of coenzyme throughout the family of pyridine nucleotide-dependent dehydro-genases. The purified GDH was inactivated in a dose-dependent manner by phenylglyoxal. Either NADH or 2-oxoglutarate did not gave any protection against the inactivation caused by a phenylglyoxal. This result indicates that GDH saturated with NADH or 2-oxoglutarate is still open to attack by phenylglyoxal. Phenylglyoxal was an uncompetitive inhibitor (K_i = 5 μM) with respect to 2-oxoglutarate and a noncompetitive inhibitor (K_i = 6 μM) with respect to NADH. The above results suggests that the phenylglyoxal-modified arginine residues are not located at the catalytic site and the inactivation of GDH by phenylglyoxal might be due to a steric hindrance or a conformational change affected by the interaction of the enzyme with its inhibitor.

Keywords: Arginine; Chemical Modification; Glutamate Dehydrogenase; Lysine; *Sulfolobus solfataricus*.

Introduction

Glutamate dehydrogenase (GDH) (EC 1.4.1.2–4) is a family of enzymes that catalyze the reversible deamination of L-glutamate to 2-oxoglutarate using NAD^+, $NADP^+$ or both as coenzymes (Fisher, 1985). Depending on the coenzyme involved in the reaction, GDH is classified into two distinct classes; the $NADP^+$-dependent enzyme which is involved in ammonia assimilation and the NAD^+-dependent enzyme which takes part in glutamate catabolism. The enzymes purified from vertebrates are able to use both the coenzymes, whereas the enzymes from microorganisms are specific for one of the pyridine nucleotides (Smith *et al*., 1975). An important difference among GDHs is the sensitivity to regulation exerted by purine nucleotides. GDHs from microorganisms are subjected to a rigid control of their cellular levels by induction-repression mechanisms and are relatively insensitive to allosteric control of their activity, whereas those from higher sources are finely tuned by a number of allosteric modulators, mainly purine nucleotides (Cho and Yoon, 1999; Cho *et al*., 1996; 1998b; Smith *et al*., 1975). Several authors have suggested similar conformations for GDHs (Blumenthal *et al*., 1975) and a common evolutionary origin (Wooton, 1974). Whereas, other GDHs such as those NAD^+-dependent enzymes isolated from *Saccharomyces cerevisiae* and *Neurospora crassa* have a

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Abbreviations: GDH, glutamate dehydrogenase; *S. solfataricus*, *Sulfolobus solfataricus.*

tetrameric assembly with subunits of 116 kDa. (Uno *et al*., 1984; Veronese *et al*., 1974). It is noteworthy that GDHs purified from various sources have showed complex regulations and allosteric interactions between subunits (Consalvi *et al*., 1991; Syed and Engel, 1984).

The discovery of extremely thermophilic archaebacteria gives rise to the hope that the enzyme proteins of these organisms could show the structural requisites for thermophilic behavior more clearly than the proteins of the rather moderately thermophilic eubateria and eukaryotes investigated up to now. The enzymes from thermophilic bacteria have aroused interest from researchers. This growing interest is basically justified by two reasons. Firstly, these molecules may contribute to a better understanding of the mechanisms of thermostability; secondly, their chemical physical properties are particularly suitable for many biotechnological processes. *Sulfolobus solfataricus*, a hyperthermophilic, sulfur-dependent microorganism living optimally at 89°C , is one of the well-studied archaebacteria (Marino *et al*., 1988; Rella *et al*., 1987) and a large biomass is easily obtained. Because *S. solfataricus* grows at very high temperatures, it should provide a source of enzymes with unusual physicochemical properties. In addition to its growth in extreme environments, the study of *S. solfataricus* as a member of the third primary kingdom of organisms (Woese *et al*., 1977) is interesting from a phylogenetic point of view.

Since the nitrogen metabolism of *S. solfataricus* is completely unknown (Jones *et al*., 1987), the abundance of GDH and its putative involvement in amino group metabolism motivated us to study this enzyme. It has been shown that *S. solfataricus* GDH might be the first enzyme involved in the biosynthesis of amino group by conversion of 2-oxoglutarate and ammonia to glutamate (Consalvi *et al*., 1991). The GDH from *S. solfataricus* presents some interesting properties; it has a double coenzyme specificity, is strictly specific for L-glutamate and 2-oxoglutarate and its thermal stability is a function of the enzyme concentration. Further interest stems from potential biotechnological applications and from a more direct approach toward the problem of protein thermostability, and evolutionary consideration. We recently investigated different types of GDH isoproteins from bovine brain (Cho *et al*., 1995) and reported the molecular properties of the GDH isoproteins (Cho and Lee, 1996; Cho *et al*., 1998a; 1999; Lee *et al*., 1998). Our work led to the finding that GDH is present in bovine brain in “heat-labile” and “heat-stable” forms (Cho *et al*., 1995). The reactive amino acids involved in catalysis of brain GDH have also been reported (Ahn *et al*., 1999; Cho and Yoon, 1999; Cho *et al*., 1996; 1998b; 1999; Kim *et al*., 1997). There is a relatively low homology between microbial and mammalian GDHs and the comparison of the detailed structure and function of the various GDH species has rarely been reported. Although three-dimensional structures of GDH from microorganisms (Baker *et al*., 1992; Yip *et al*., 1995) and mammalian sources (Peterson *et al*., 1997) are available, there are uncertainties regarding the catalytic mechanism and the role played by the various effectors on the enzyme.

In the present study, characterization and chemical modification studies of the specific *S. solfataricus* GDH have been undertaken with the aim of contributing to the study of structure-function and evolutionary relationships between various types of GDHs.

Materials and Methods

Materials *Sulfolobus solfataricus*, strain MT-4 was purchased at ATCC (American Type Culture Collection). NADH, ADP, GTP, ammonium acetate, and 2-mercaptoethanol, L-leucine, 2-oxoglutarate, L-glutamate, phenylmethylsulfonyl fluoride, o-phthalaldehyde, phenylglyoxal, diethylpyrocarbonate, and EDTA were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Q-Sepharose and Blue Sepharose were purchased from Pharmacia. Hydroxyapatite was obtained from Bio-Rad. Matrix gel red-A and ultrafiltration Diaflo membranes were from Amicon Corp. Pre-cast gels for SDS-polyacrylamide gel electrophoresis were purchased from NOVEX. Low molecular-mass standards for SDS/PAGE were purchased from Bio-Rad. All other chemicals and solvents were reagent grade or better.

Growth conditions of *S. solfataricus* Briefly, *S. solfataricus*, strain MT-4 was grown aerobically at 87°C and pH 3.5 as previously described (Rosa *et al*., 1984) using 0.2% yeast extract as nutrient. Minimal growth media contained 1% casamino acid. Cells were harvested in the stationary growth phase by continuous shaking using Lab-line orbit environ-shaker.

Enzyme purification and assay GDH was purified from *S. solfataricus* by the method described by Consalvi *et al*. (1991) and tested for homogeneity using Coomassie-stained gradient SDS-polyacrylamide gel electrophoresis. Glutamate dehydrogenase activity was measured spectrophotometrically in the direction of reductive amination of 2-oxoglutarate by following the decrease in absorbance at 340 nm at 60°C. All assays were performed twice, and initial velocity data were correlated with a standard assay mixture containing 50 mM triethanolamine, pH 8.0, 100 mM ammonium acetate, 0.1 mM NADH, and 2.6 mM EDTA. GDH concentrations were adjusted to give a measured rate of less than 0.04 absorbance units/min. The reaction was started by adding 2-oxoglutarate to final concentration of 10 mM. One unit of enzyme was defined as the amount of enzyme required to oxidize 1 μmol of NADH per min under the standard assay conditions described above. Unless otherwise specified, highly purified GDH fractions were used for this study. Stimulation or inhibition studies with ADP, L-leucine, and GTP were performed at various concentrations in assay buffer as described in the table legends.

Chemical modification of GDH with o-phthalaldehyde or phenylglyoxal Solutions of o-phthalaldehyde were freshly prepared daily as described by Ahn *et al*. (1999) and Huynh

(1990). Solution of phenylglyoxal was prepared by dissolving the solid in a minimum volume of methanol and then adding water to give a 20 mM solution. The concentration of phenylglyoxal was determined from the absorbance in methanol ($\varepsilon_{247\,nm}$ = 11300 $M^{-1}cm^{-1}$) as described elsewhere (Kohlbrenner and Cross, 1978). The modification was carried out at 60°C by incubating the enzyme (10 μM) with o-phthalaldehyde or phenylglyoxal in 0.1 M potassium phosphate buffer, pH 7.4. The final concentration of methanol in the incubation mixture was no more than 1% (v/v) and was found to have no effect on enzyme activity. For inactivation studies, the enzymes were incubated with different concentrations of o-phthalaldehyde. At different time intervals, aliquots were withdrawn from the mixture to determine the remaining activity. Controls without inhibitor were included and the remaining activity is expressed as percentage of each control activity. For protection experiments, the enzymes were preincubated with varying concentrations of 2-oxoglutarate or NADH prior to the addition of phenylglyoxal. Aliquots were withdrawn from the mixture to determine the remaining activity. For kinetic studies, the assays were carried out by varying the substrate under investigation while keeping the other substrate and reagents at the optimal concentration indicated above. Initial velocity data were fitted by a least-squares method to the double-reciprocal plots.

Results and Discussion

The specific activity of the purified GDH from *S. solfataricus* was 83 units/mg, 166-fold that of the crude extract, and the molecular mass of the enzyme subunit was 45 kDa as estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 1), which is in good agreement with previously reported values (Consalvi *et al*., 1991). The purified GDH at 1 mg/ml protein concentration showed remarkable thermostability and retained more than 95% of the initial activity after incubation at −20°C, 4°C, and 25°C for up to 6 months. Unlike GDH from bovine brain (Cho *et al*., 1995), GDH from *S. solfataricus* did not suffer a considerable loss of activity upon freezing or lyophilizations and retained more than 90% of the initial activity. It has been documented that there are big differences between GDHs from mammilla and microorganisms in their regulation by allosteric effectors such as ADP, GTP, and leucine (Cho and Yoon, 1999; Cho *et al*., 1996; 1998b; Fisher, 1985; Smith *et al*., 1975). Therefore, we undertook the task of accounting for the regulatory properties of GDH from *S. solfataricus*. The activity of GDH from *S. solfataricus*, unlike GDH from bovine brain, was not significantly affected by the presence of various effectors such as ADP, GTP, and leucine (Table 1). These results are consistent with previous reports that GDHs from microorganisms are relatively insensitive to allosteric control of their activity (Smith, 1975). It should be noted that comparison of the amino acid sequence of GDH from *S. solfataricus* with those from different sources gives an overall identity of 9.2% and shows a symmetrical evolutionary distance of this archaebacterial protein from the two groups of vertebrate on one side and eubacterial and low eukaryote enzymes on the other side (Maras *et al*., 1992). This seems to confirm the presence of different evolutionary pathways in the GDH class, probably due to the appearance of enzymes characterized by a less stringent requirement for the type of pyridine coenzyme and by the lack of regulation of purine nucleotides and other reagents. Three-dimensional structural determination is obviously necessary to unequivocally determine the specific contribution to the regulatory site of amino acid and the understanding of the nature about the enzymes.

Chemical modification studies can make a useful contribution, by providing insight on what is likely to be in the highly conserved region of the sequence, and by providing a means of detecting substrate binding through protection studies. A modification of histidine residues of the purified GDH by diethylpyrocarbonate did not affect the enzyme activity (Table 1), suggesting that histidine residues are not directly involved in the catalysis of this

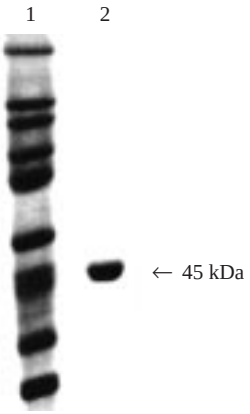

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified GDH from *S. Solfataricus*. 1, molecular weight marker proteins (Bio-Rad); 2, GDH.

Table 1. Effects of several effectors on the activity of GDH from *S. Solfataricus.* The enzyme assay was carried out as described in Materials and Methods. The values are expressed as a percentage of the control and calculated at the highest effector concentration.

Effectors	Concentration (mM)	Relative activity (%)
Control		100
ADP	0.10–10.0	90
GTP	0.01–0.10	98
Leucine	1.00–10.0	103
EDTA	0.10–10.0	100
Diethylpyrocarbonate	0.05–10.0	98
o-Phthalaldehyde	0.05	40
Phenylglyoxal	4.0	25

enzyme. o-Phthalaldehyde, a homobifunctional cross-linking reagent, has been used as an active-site inhibitor of many enzymes containing SH and NH_2 groups about 3Å apart (Huynh, 1990; Rider *et al*., 1989). Most previous work suggests that o-phthalaldehyde forms an isoindole adduct by cross-linking the ε-amino group and the sulfhydryl group of the active site lysine and cysteine residues, respectively (Ahn *et al*., 1999; Pandey *et al*., 1996). The effect of o-phthalaldehyde on the reaction rate of GDH has been investigated. The purified GDH was inactivated by o-phthalaldehyde at 60°C. Incubation of GDH with increasing concentration of o-phthalaldehyde resulted in a progressive decrease in enzyme activity (Fig. 2). After 5 min of incubation with 100 μM o-phthalaldehyde, more than 98% of the original activity of the GDH was lost, suggesting that the o-phthalaldehyde modified lysine or cysteine residue is directly involved in catalysis. A more detailed analysis of the effect of o-phthalaldehyde on GDH showed that the inhibition was competitive with respect to 2-oxoglutarate (K_i = 30 μM) at a fixed concentration of NADH, and o-phthalaldehyde also behaved as a competitive inhibitor with respect to NADH (K_i = 100 μM) at fixed 2-oxoglutarate (Fig. 3). These results further support a possibility that the o-phthalaldehyde modified residues may be involved at the catalytic, although the three-dimensional structure of GDH from *S. solfataricus* is not available yet.

Chemical modification studies have shown the existence of the essential lysines of GDH from various sources. Particularly, several lysines have been shown to have specific roles. Many of the studies have shown the Lys-126 labeled with pyridoxal 5′-phosphate is the essential lysine residue in mammallian GDH reaction (Chen and Engel, 1975; Kim *et al*., 1997; McPherson *et al*., 1988). Using trinitrobenzene sulfonic acid, however, it was reported that Lys-422 is involved in the catalytic activity, as well as the regulation and maintenance of quaternary structure, while Lys-419 only has a structural role (Coffee *et al*., 1971; Goldin *et al*., 1971). Talbot *et al*. (1977) showed evidence for the existence of two pyridoxal 5′-phosphate binding sites, Lys-126 and Lys-333. Recently, Pandey *et al*. (1996) reported that bovine liver GDH is inactivated by o-phthalaldehyde and that cysteine and lysine residues are present at the active site. Very recently we proposed (Ahn *et al*., 1999) that Lys-306 of the bovine brain GDH is the site of o-phthalaldehyde modification in the peptide backbone between Leu-296 and Lys-308. It was also suggested (Ahn *et al*., 1999) that the o-phthalaldehyde-modified lysine residue is different from the previously reported reactive lysine residues such as Lys-126, Lys-419,

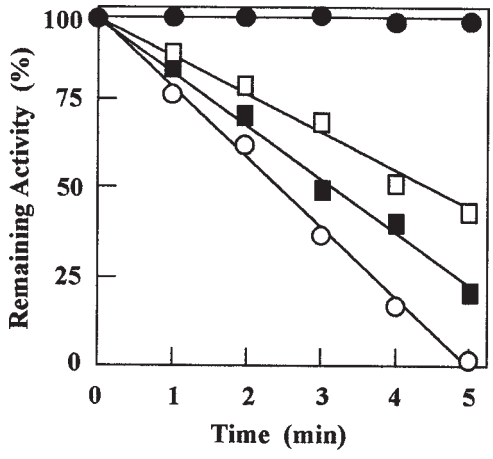

Fig. 2. Time-dependent inactivation of GDH by o-phthalaldehyde. GDH was incubated with the indicated concentrations of o-phthalaldehyde in the assay mixture at 60°C. After 2 min, aliquots were withdrawn and the activity was assayed by the addition of the standard assay mixture. Remaining activities are expressed as a percentage of each control. The concentrations of o-phthalaldehyde used were 0 μM (●), 25 μM (□), 50 μM (■), and 100 μM (○).

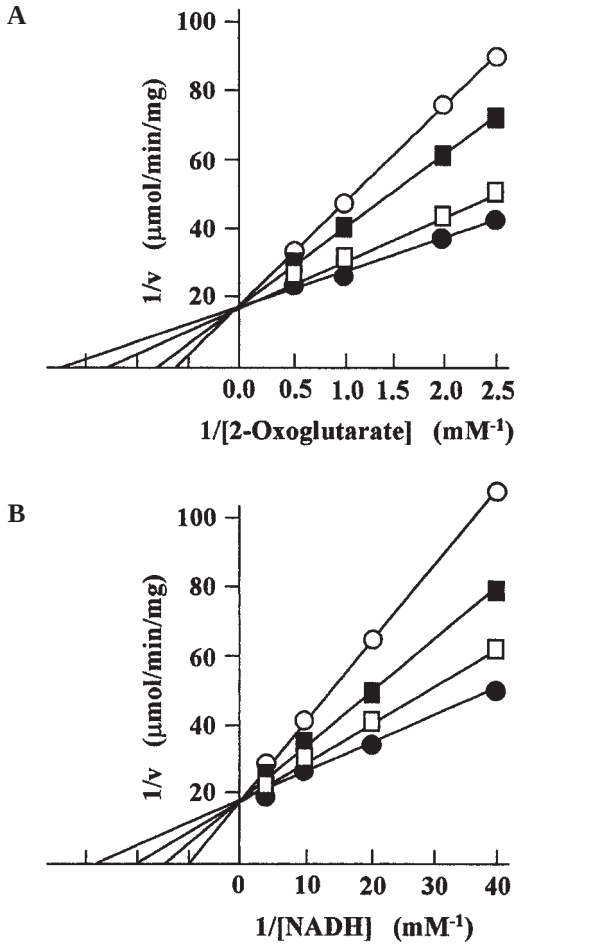

Fig. 3. Inhibition of GDH activity by o-phthalaldehyde. **A.** The concentration of NADH (100 μM) was kept constant and that of 2-oxoglutarate was varied. **B.** The concentration of 2-oxoglutarate (10 μM) was kept constant and that of NADH was varied. Results were obtained at concentrations of o-phthalaldehyde of 0 μM (●), 50 μM (□), 100 μM (■), and 150 μM (○) at pH 8.0.

and Lys-422 by other labeling studies (Coffee *et al*., 1971; Goldin *et al*., 1971; Kim *et al*., 1997). The three-dimensional structure of GDH from *Clostridium symbiosum* suggests that Lys-306 would appear to be in a loop joining β-j and β-k, some distance from the coenzyme binding surface of the NAD^+-domain and still further from the sites for catalysis and glutamate binding. A comparison of the detailed structure and function of the various GDH species rarely has been reported. The detailed primary structure of the o-phthalaldehyde-modified residue in the overall sequence of GDH from *S. solfataricus* remains to be determined.

The modification of GDH by the arginine-specific dicarbonyl reagent phenylglyoxal was examined with the view that arginine residues might play a general role in the binding of coenzyme throughout the family of pyridine nucleoide-dependent dehydrogenases (Foster and Harrison, 1974; Lange *et al*., 1974; Yang and Schwert, 1972). The common feature of the enzymes for which arginine is essential seems to be the catalysis of a reaction involving a negatively charged substrate or coenzyme. Since GDH has negatively charged substrates and coenzymes, it has several regions that might be postulated to include arginine. Actually, it was reported that functional arginine residues are involved in coenzyme binding by *Neurospora* GDH (Blumenthal and Smith, 1975). The purified GDH from *S. solfataricus* was inactivated in a dose-dependent manner by phenylglyoxal at 60°C as shown in Fig. 4. Either NADH or 2-oxoglutarate did not gave any protection against the inactivation caused by a phenylglyoxal (Fig. 4). No significant protection was observed by the combined addition of NADH and 2-oxoglutarate (data not shown). This result indicates that GDH saturated with NADH or 2-oxoglutarate is still open to attack by phenylglyoxal. The data presented in Fig. 5 indicate that phenylglyoxal is an uncompetitive inhibitor (K_i = 5 mM) with respect to 2-oxoglutarate at fixed concentrations of NADH and a noncompetitive inhibitor (K_i = 6 mM) with respect to NADH at fixed concentrations of 2-oxoglutarate. The above results suggest that the phenylglyoxal-modified arginine residues are not located at the coenzyme or the substrate binding site and the inactivation of GDH by phenylglyoxal may be due to a steric hindrance or a conformational change effected by the interaction of the enzyme with its inhibitor.

To our knowledge, this is the first report showing an involvement of reactive lysine or cysteine residues in the catalysis of *S. solfataricus* GDH. GDH protein may provide an explanation for nitrogen metabolism in archaebacteria and offer new perspectives in understanding enzyme thermostability, and may reveal more information

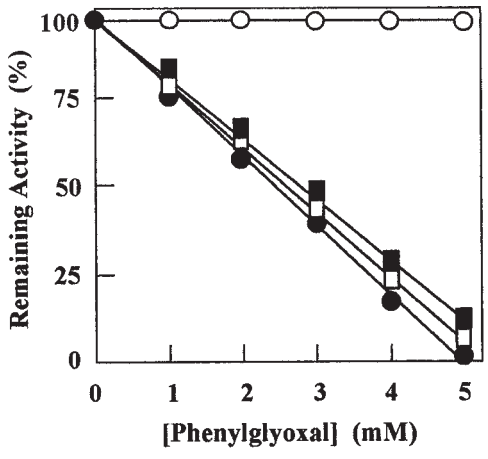

Fig. 4. Dose-dependent inactivation of GDH by phenylglyoxal. GDH was incubated with the indicated concentrations of phenylglyoxal in the presence of NADH or 2-oxoglutarate at 60°C. After 5 min, aliquots were withdrawn and the activity was assayed by the addition of the standard assay mixture. Remaining activities are expressed as percentage of each control. ●, (GDH + phenylglyoxal); □, (GDH + NADH + phenylglyoxal); ■, (GDH + 2-oxoglutarate + phenylglyoxal); ○, (GDH only).

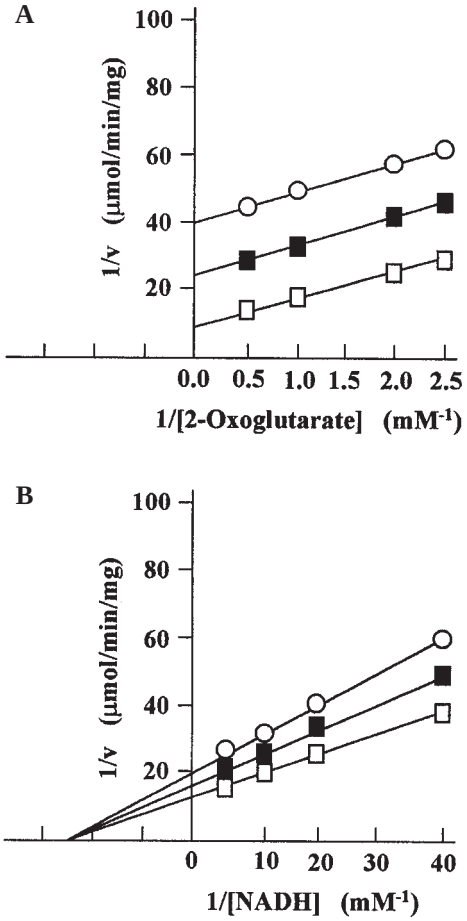

Fig. 5. Inhibition of GDH activity by phenylglyoxal. **A.** The concentration of NADH (100 μM) was kept constant and 2-oxoglutarate was varied. **B.** The concentration of 2-oxoglutarate (10 mM) was kept constant and NADH was varied. Results were obtained at concentrations of phenylglyoxal of 1 mM (□), 2 mM (■), and 3 mM (○) at pH 8.0.

about the evolution of this enzyme during the progression towards the eukaryotes. This information is needed to establish the possible evolutionary relationship between *S. solfataricus* GDH and other well-known eukaryotic GDH. Therefore, further studies are required to elucidate the physiological roles of various types of the GDH proteins. Further sequence characterization of *S. solfataricus* GDH is in progress in our laboratory.

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References

Ahn, J.-Y., Choi, S. Y., and Cho, S.-W. (1999) Identification of lysine residue involved in inactivation of brain glutamate dehydrogenase isoproteins by o-phthalaldehyde. *Biochimie* **81**, 1123–1129.

Baker, P. J., Britton, K. L., Rice, D. W., Rob, A., and Stillman, T. J. (1992) Structural consequences of sequence patterns in the fingerprint region of the nucleotide binding fold implications for nucleotide specificity. *J. Mol. Biol.* **228**, 662–671.

Blumenthal, K. M. and Smith, E. L. (1975) Functional arginine residues involved in coenzyme binding by glutamate dehydrogenases. *J. Biol. Chem.* **250**, 6555–6559.

Blumenthal, K. M., Moon, K. E., and Smith, E. L. (1975) Nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase of *Neurospora*. *J. Biol. Chem.* **250**, 3644–3654.

Chen, S.-S. and Engel, P. C. (1975) The equilibrium position of the reaction of bovine liver glutamate dehydrogenase with pyridoxal 5′-phosphate. A demonstration that covalent modification with this reagent completely abolishes catalytic activity. *Biochem. J.* **147**, 351–358.

Cho, S.-W. and Lee, J. E. (1996) Modification of brain glutamate dehydrogenase isoproteins with pyridoxal 5′-phosphate. *Biochimie* **78**, 817–821.

Cho, S.-W., Lee, J., and Choi, S. Y. (1995) Two soluble forms of glutamate dehydrogenase isoproteins from bovine brain. *Eur. J. Biochem*. **233**, 340–346.

Cho, S.-W., Ahn, J.-Y., Lee, J., and Choi, S. Y. (1996) Identification of a peptide of the guanosine triphosphate binding site within brain glutamate dehydrogenase isoproteins using 8-azidoguanosine triphosphate. *Biochemistry* **35**, 13907–13913.

Cho, S.-W., Cho, E. H., and Choi, S. Y. (1998a) Activation of two types of brain glutamate dehydrogenase isoproteins by gabapentin. *FEBS Lett.* **426**, 196–200.

Cho, S.-W., Yoon, H.-Y., Ahn, J.-Y., Choi, S. Y., and Kim, T. U. (1998b) Identification of an NAD^+ binding site of brain glutamate dehydrogenase isoproteins by photoaffinity labeling. *J. Biol. Chem*. **272**, 31125–31130.

Cho, S.-W. and Yoon, H.-Y. (1999) Photoaffinity labeling of brain glutamate dehydrogenase isoproteins with an azido-ADP. *J. Biol. Chem.* **274**, 13948–13953.

Cho, S.-W., Cho, E. H., Hwang, S.-H., and Choi, S. Y. (1999) Reactive cysteine residue of Bovine Brain Glutamate Dehydrogenase Isoproteins. *Mol. Cells* **9**, 91–98.

Choi, S. Y., Hong, J. W., Song, M.-S., Jeon, S. G., Bahn, J. H., Lee, B. Y., Ahn, J.-Y., and Cho, S.-W. (1999) Different antigenic reactivities of bovine brain glutamate dehydrogenase isoproteins. *J. Neurochem*. **72**, 2162–2169.

Coffee, C. J., Bradshaw, R. A., Goldin, B. R., and Frieden, C. (1971) Identification of the sites of modification of bovine liver glutamate dehydrogenase reacted with trinitrobenzenesulfonate. *Biochemistry* **10**, 3516–3526.

Consalvi, V., Chiaraluce, R., Politi, L., Gambacorta, A., Rosa, M., and Scandurra, R. (1991) Glutamate dehydrogenase from the thermoacidophilic archaebacterium *Sulfolobus solfataricus*. *Eur. J. Biochem*. **196**, 459–467.

De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio, E., and Buonocore, V. (1984) Glucose metabolism in the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus*. *Biochem. J.* **224,** 407–414.

Fisher, H. F. (1985) L-Glutamate dehydrogenase from bovine liver. *Methods Enzymol*. **113**, 16–27.

Foster, M. and Harrison, J. H. (1974) Selective chemical modification of arginine residues in mitochondrial malate dehydrogenase. *Biochem. Biophys. Res. Commun*. **58**, 263–267.

Goldin, B. R. and Frieden, C. (1971) L-Glutamate dehydrogenases. *Curr. Top. Cell. Reg*. **4**, 77–117.

Huynh, Q. K. (1990) Mechanism of inactivation of *Escherichia coli* 5-enolpyruvoylshikimate-3-phosphate synthase by o-phthalaldehyde. *J. Biol. Chem.* **265**, 6700–6704.

Jones, W. J., Nagle, D. P., and Whitman, W. B. (1987) Methanogens and the diversity of archaebacteria. *Microbiol. Rev*. **51**, 135–177.

Kim, S. W., Lee, J., Song, M. S., Choi, S. Y., and Cho, S.-W. (1997) Essential active-site lysine of brain glutamate dehydrogenase isoproteins. *J. Neurochem*. **69**, 418–422.

Kohlbrenner, W. E. and Cross, R. L. (1978) Efrapeptin prevents modification by phenylglyoxal of an essential arginyl residue in mitochondrial adenosine triphosphatase. *J. Biol. Chem.* **253**, 7609–7611.

Lange, L. G., Riordan, J. F., and Vallee, B. L. (1974) Functional arginyl residues as NADH binding sites of alcohol dehydrogenases. *Biochemistry* **13**, 4361–4370.

Lee, J. E., Choi, S. Y., and Cho, S.-W. (1998) Expression and localization of brain glutamate dehydrogenase with its monoclonal antibody. *Kor. J. Biol. Sci*. **2**, 71–80.

Maras, B., Consalvi, V., Chiaraluce, R., Politi, L., De Rosa, M., Bossa, F., Scandurra, R., and Barra, D. (1992) The protein sequence of glutamate dehydrogenase from *Sulfolobus solfataricus*, a thermoacidophilic archaebacterium. *Eur. J. Biochem*. **203**, 81–87.

Marino, G., Nitti, G., Arnone, M. I., Sannia, G., Gambacorta, A., and De Rosa, M. (1988) Purification and characterization of aspartate aminotransferase from the thermoacidophilic archaebacterium *Sulfolobus solfataricus*. *J. Biol. Chem.* **263**, 12305–12309.

McPherson, M. J., Baron, A. J., Jones, K. M., Price, G. J., and Wootton, J. C. (1988) Multiple interactions of lysine-128 of *Escherichia coli* glutamate dehydrogenase revealed by site-directed mutagenesis studies. *Protein. Eng*. **2**, 147–152.

Pandey, A., Sheikh, S., and Katiyar, S. S. (1996) Identification of cysteine and lysine residues present at the active site of beef liver glutamate dehydrogenase by o-phthalaldehyde. *Biochim. Biophys. Acta* **1293**, 122–128.

Peterson, P. E., Pierce, J., and Smith, T. J. (1997) Crystallization and characterization of bovine liver glutamate dehydrogenase. *J. Struct. Biol*. **120**, 73–77.

Rella, R., Raia, C. A., Pensa, M., Pisani, F. M., Gambacorta, A., De Rosa, M., and Rossi, M. (1987) A novel archaebacterial NAD^+-dependent alcohol dehydrogenase. Purification and properties. *Eur. J. Biochem*. **167**, 475–479.

Rider, R. N. and Hue, L. (1989) Inactivation of liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase by o-phthalaldehyde. *Biochem. J.* **262**, 97–102.

Smith, E. L., Austen, B. M., Blumenthal, K. M., and Nyc, J. F. (1975) Glutamate dehydrogenase; in *The Enzymes*, 3rd ed, P. D. Boyer (ed.) Vol. XI, pp. 293–367, Academic Press, New York.

Syed, S. E.-H. and Engel, P. C. (1984) Ox liver glutamate dehydrogenase. The use of chemical modification to study the relationship between catalytic sites for different amino acid substrates and the question of kinetic non-equivalence of the subunits. *Biochem. J*. **222**, 621–626.

Talbot, J.-C., Gros, C., Cosson, M.-P., and Pantaloni, D. (1977) Physiochemical evidence for the existence of two pyridoxal 5′-phosphate binding sites on glutamate dehydrogenase and characterization of their functional role. *Biochim. Biophys. Acta* **494**, 19–32.

Uno, I., Matsumoto, K., Adachi, K., and Ishikawa, T. (1984) Characterization of cyclic AMP-requiring yeast mutants altered in the catalytic subunit of protein kinase. *J. Biol. Chem*. **259**, 12508–12513.

Veronese, F. M., Nyc, J. F., Degani, Y., Brown, D. M., and Smith, E. L. (1974) Nicotinamide adenine dinucleotide-specific glutamate dehydrogenase of Neurospora. I. Purification and molecular properties. *J. Biol. Chem.* **249**, 7922–7928.

Woese, C. R. (1987) Bacterial evolution. *Microbiol Rev*. **51**, 221–271.

Wootton, J. C. (1974) The coenzyme-binding domains of glutamate dehydrogenases. *Nature* **252**, 542–546.

Yang, P. C. and Schwert, G. W. (1972) Inactivation of lactate dehydrogenase by butanedione. *Biochemistry* **11**, 2218–2224.

Yip, K. S. P., Stillman, T. J., Britton, K. L., Artymiuk, P. J., Baker, P. J., Sedelnikova, S. E., Engel, P. C., Pasquo, A., Chiaraluce, R., Consalvi, V., Scandurra, R., and Rice, D. W. (1995) The structure of Pyrococcus furiosus glutamate dehydrogenase reveals a key role for ion-pair networks in maintaining enzyme stability at extreme temperatures. *Structure* **3**, 1147–1158.