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NOTE

Glyoxal Detoxification in *Escherichia coli* K-12 by NADPH Dependent Aldo-keto Reductases

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Glyoxal (GO) and methylglyoxal (MG) are reactive carbonyl compounds that are accumulated *in vivo* through various pathways. They are presumably detoxified through multiple pathways including glutathione (GSH)-dependent/independent glyoxalase systems and NAD(P)H dependent reductases. Previously, we reported an involvement of aldo-ketoreductases (AKRs) in MG detoxification. Here, we investigated the role of various AKRs (YqhE, YafB, YghZ, YeaE, and YajO) in GO metabolism. Enzyme activities of the AKRs to GO were measured, and GO sensitivities of the corresponding mutants were compared. In addition, we examined inductions of the AKR genes by GO. The results indicate that AKRs efficiently detoxify GO, among which YafB, YghZ, and YeaE are major players.

***Keywords*:** glyoxal, methylglyoxal, aldo-keto reductase

Glyoxals are 2-oxoaldehydes present in various cell types, including prokaryote and eukaryote. These are reactive dicarbonyls accumulated through various pathways, such as protein glycation, lipid peroxidation, DNA oxidation, and sugar autoxidation (Thornalley *et al.*, 1999). Glyoxals interact with nucleic acids and proteins, thereby causing protein function and increasing mutation frequency via DNA adducts (Kasai *et al.*, 1998; Rondeau and Bourdon, 2011). It has been reported that diseases, such as diabetes, Alzheimer, and Parkinson, are associated with an accumulation of these toxic aldehydes (Brownlee, 1995; Munch *et al.*, 2000; Vander Jagt and Hunsaker, 2003; Luth *et al.*, 2005). Detoxification of glyoxals involves the glutathione (GSH)-dependent glyoxalase system (GloI and II) and various reductases, including aldo-keto reductases (AKRs; Fig. 1.) (Thornalley, 1990; Vander Jagt *et al.*, 1992; Thornalley, 1998; Ko *et al.*, 2005). Recently, the GSH-independent glyoxalase III has been characterized (Fig. 1) (Subedi *et al.*, 2011).

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We have previously characterized the YqhD aldehyde reductase that catalyzes GO to 1,2-ethandiol via glycolaldehyde (GA) using NADPH (Fig. 1) (Lee *et al.*, 2010). In addition, we found that although the GSH-dependent glyoxalase system plays a major role in MG detoxification, this enzyme is not primarily involved in GO detoxification (Lee *et al.*, 2010). This suggests that there might be some other pathways for detoxifying GO other than the GSH-dependent glyoxalases, i.e. the YqhD aldehyde reductase.

Our search for homologs of the mammalian AKRs, known to exhibit activity to MG, revealed nine genes from the *E. coli* genome (Ko *et al.*, 2005). Based on assays of enzyme activity for crude extracts from *E. coli* mutant strains and for purified proteins, we selected five genes, *yafB*, *yqhE*, *yeaE*, *yghZ*, and *yajO*, for further characterization (Ko *et al.*, 2005). In this experiment, MG susceptibilities of the AKR mutants were only observed in the GloI-deficient background (Ko *et al.*, 2005). So far, role of AKRs in GO metabolism has not been experimentally demonstrated. In this study, we measured enzymatic activities of purified AKRs (YafB, YqhE, YeaE, YghZ, and YajO). In addition, GO susceptibilites of the AKR-deficient mutants were observed by measuring inhibitory concentrations for GO and cellular viabilities to GO, suggesting an importance of NADPH-dependent AKRs in GO detoxification. Induction of AKR genes by GO was also monitored by quantitative RT-PCR.

According to the previous study (Ko *et al.*, 2005), the YafB, YqhE, YeaE, and YghZ enzymes convert MG to acetol by using NADPH with specific activities of 14900, 21000, 1890, and 2200 nmole/min/mg for MG (1 mM), respectively. However, the YajO protein did not show an apparent enzymatic activity (Specific activity of 1 mM MG less than 10 nmole/min/mg) (Ko *et al.*, 2005). We measured enzyme activities of AKRs for GO and GA spectrophotometrically by monitoring oxidation of a cofactor, NADPH. GO can be reduced to GA and further to 1,2-ethandiol by YqhD aldehyde reductase (Fig. 1, Table 1) (Lee *et al.*, 2010). When we examined activities of the purified AKR enzymes, YafB and YqhE exhibited fairly high activities to GO (Table 1). Although the enzymatic activities of YghZ and YeaE for GO were much lower, the GA-converting activity of YghZ was significantly greater than that of the other AKRs. In the case of YeaE, K_m values for both GO and GA are fairly low. As in the previous report on MG, YajO shows enzymatic activity to neither GO nor GA. Other enzymatic parameters, such as K_m, k_{cat} and k_{cat} / K_m, are described in Table 1.

When we tested GO susceptibilities of *E. coli* strains de-

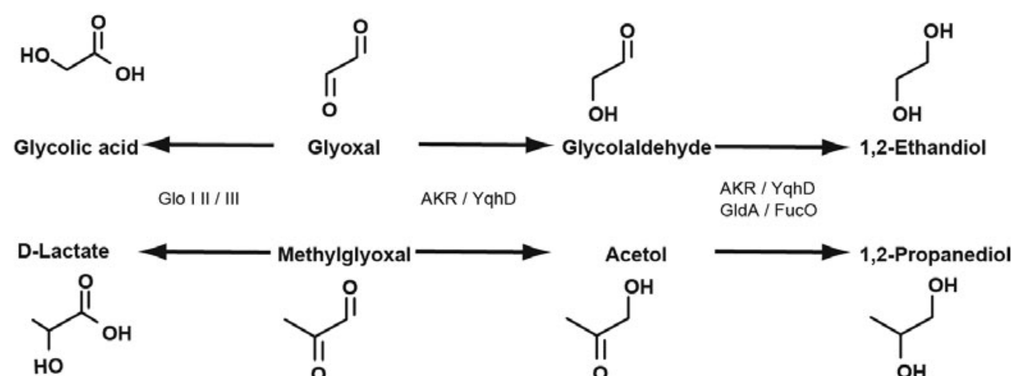

Fig. 1. Detoxification pathways for glyoxals. Glyoxals, such as GO and MG, can be detoxified by several pathways involving a number of enzymes listed here. The GSH-dependent glyoxalase system (GloI and II) converts GO and MG into glycolic acid and D-lactate, respectively (Thornalley, 1990). GloIII also generates lactate without the use of any additional cofactor (Subedi *et al.*, 2011). In addition, various enzymes, including AKR, YqhD, GldA, and FucO, are involved in reduction of glyoxals to corresponding alcohols using NAD(P)H (Cocks *et al.*, 1974; Boronat and Aguilar, 1979; Baldoma and Aguilar, 1987; Zhu and Lin, 1989; Altaras and Cameron, 1999; Ko *et al.*, 2005; Lee *et al.*, 2010).

fective in AKR genes, they showed similar sensitivity to that of *yqhD* mutant, except for *yqhE* and *yajO* mutants. To compare IC_{50} among strains, we measured optical densities of cells growing in media containing different concentrations of GO as described previously (Lee *et al.*, 2010). IC_{50}s obtained are Δ*yqhD* (1.88 ± 0.14 mM), Δ*yafB* (2.42 ± 0.21), Δ*yghZ* (2.48 ± 0.19), Δ*yeaE* (2.41 ± 0.18), wild type (2.87 ± 0.20), Δ*yqhE* (2.78 ± 0.22), and Δ*yajO* (2.92 ± 0.20). Consistant with the IC_{50}s of mutants, the GO-induced lethalities of *yafB*, *yghZ*, and *yeaE* mutants are less severe than that of the *yqhD* mutant (Fig. 2). Susceptibilities to GA were basically similar in the strains tested including wild type. As described previously, the AKR mutants did not show apparent sensitivities to MG in $gloA^+$ background, suggesting that the AKRs are critical in GO detoxification but are less important in MG detoxification, and that reduction of GO, rather than GA, is influential in protecting cells against GO.

We observed GO-dependent induction of AKR genes by performing qRT-PCR. *E. coli* MG1655 cells at OD_{600} of 1 were treated with 1 and 5 mM of GO for 30 min. Subsequently, RNA was isolated, which was used as a template to synthesize cDNA. qRT-PCR was performed using primers designed to recognize the AKR genes (Fig. 3). The *yqhE* gene was highly expressed even in the absence of GO relative to that of other AKRs. Furthermore, *yqhE* was responsive to GO. In contrast to *yqhE*, *yghZ*, and *yajO* expressions were decreased in dose-dependent manner with GO treatment. The *yafB* and *yeaE* genes were not induced by GO, whose expression levels were relatively lower than that of other AKRs.

While the intracellular detoxification of MG occurs through multiple pathways including the GSH-mediated glyoxalase I/II system, pathways for GO are not well established. We observed that GO sensitivity of *gloA*-deficient strain was not much different from that of wild type (Lee *et al.*, 2010), suggesting the presence of other pathways for intracellular GO since the GSH-dependent glyoxalase system is not the major pathway. Cells expressing the YqhD enzyme may be advantageous in GO detoxification, since it is capable of converting a toxic GO to nontoxic 1,2-ethandiol by a single enzyme. As a matter of fact, the aldo-keto reductase YghZ, showing considerable activity to both GO and GA (Table 1), plays a significant role in *in vivo* detoxification of GO (Fig. 2). YafB and YeaE are also involved in GO detoxification *in vivo* (Fig. 2). YafB protein exhibits fairly good enzyme activity *in vitro*. Even though the catalytic activity of YeaE

Table 1. Enzymatic constants for purified aldo-keto reductases

Substrates	Glyoxal				Glycolaldehyde			
Proteins[a]	Specific activity (nmole/min/mg)	K_m (mM)	k_{cat} (min^{-1})	k_{cat} / K_m ($min^{-1}M^{-1}$)	Specific activity (nmole/min/mg)	K_m (mM)	k_{cat} (min^{-1})	k_{cat} / K_m ($min^{-1}M^{-1}$)
YqhD[b]	36,250	11	618	56,182	61,902	28	3,258	1,116,357
YqhE	12,400	22±2	296±15	13,455	620	12±0.6	15±0.6	1,250
YafB	17,800	60±4	368±20	6,133	790	17±1	22±2	1,294
YghZ	8,801	104±7	458±18	4,404	2,247	104±6	140±9	1,346
YeaE	357	10±0.4	15±0.5	1,500	306	7±0.3	15±0.5	2,143
YajO[c]	ND	ND	ND	ND	ND	ND	ND	ND

[a] The AKR genes were cloned in pET21b (Novagen) and expressed in BL21(DE3) strain. The AKR proteins were purified with Ni-NTA column following the standard procedure (Novagen). Enzyme activity of the purified AKRs was measured at 25°C using a Beckman Coulter DU800 spectrophotometer by monitoring the initial rate at 340 nm for an oxidation of NADPH. The substrate specificity of the 2 μg protein was assessed in 1 ml of 50 mM potassium phosphate buffer (pH 7.0) with 0.1 mM NADPH as a coenzyme. Concentrations of the substrates used to measure specific activity were 200 mM of glyoxal and glycolaldehyde.
[b] 2 mM of NADPH and 10 μg purified protein was used for YqhD (Lee *et al.*, 2010).
[c] Enzyme activity of YajO was not detected (ND).

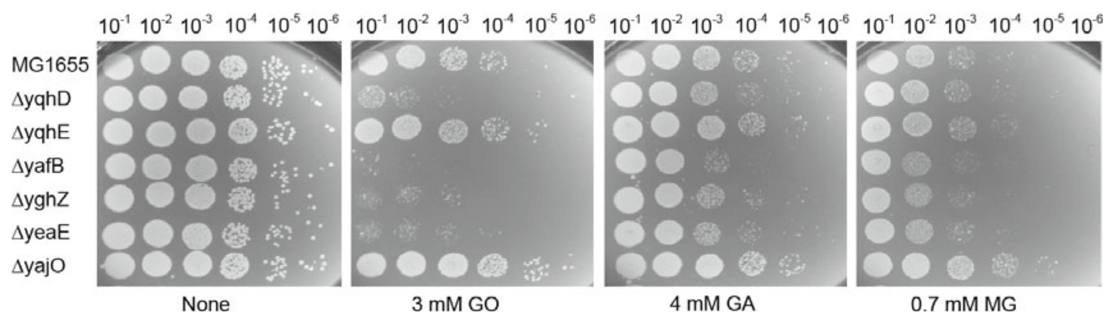

Fig. 2. Sensitivities of AKR mutants to GO, GA, and MG. Cell viability after being exposed to aldehyde compounds was determined in order to assess their toxic effects by growing cells on LB plate containing different concentrations of compounds (Lee *et al.*, 2010). Among the AKR deficient strains, Δ*yafB*, Δ*yghZ*, and Δ*yeaE* show sensitivity to GO. The GO susceptibilities of Δ*yafB*, Δ*yghZ*, and Δ*yeaE* are comparable to that of Δ*yqhD*. Effects of GA and MG on survival were also tested, but difference in sensitivity was not observed among the mutants. All strains used are derivatives of *E. coli* K-12. MG1655 was used as a wild-type strain for gene disruption (Ko *et al.*, 2005).

protein is low for GO and GA, it might be effective in cytosol because of its low K_m values for both aldehydes. In the case of YqhE, the enzyme activity for GO is considerably high with high level expression relative to other AKRs. However, *yqhE* mutant does not become susceptible to GO. Thus, there might be some unknown factor in *in vivo* function of YqhE.

Previously, coregulation of *yqhE* and its adjacent *yqhD* gene had been reported (Turner *et al.*, 2011). In addition, it was also shown that YqhC as a transcriptional activator regulates *yqhD* expression by direct binding to the promoter region of *yqhD* (Lee *et al.*, 2010). Thus, it is likely that GO induction of *yqhE* is mediated by YqhC. YafB is known to be negatively regulated by NsrR and FNR transcriptional factors (Kwon *et al.*, 2012). These regulators are involved in sensing nitric oxide (NO) and aerobic condition, respectively, through their iron-sulfur (2Fe-2Se) clusters (Salmon *et al.*, 2003; Tucker *et al.*, 2010). Interestingly, expressions of *yghZ* and *yajO* were decreased upon GO exposure. However, regulation mechanism of these genes is still unknown. Novel regulatory mechanisms responding GO or GO-induced signals might exist. It is likely that YghZ and YajO respond to low concentration of GO in physiological condition. Even though the role of AKRs in GO metabolism is thought to be redundant, since these AKRs have quite different kinetic and expression properties regarding GO, they might have their unique roles in GO detoxification in response to various physiological conditions, i.e. concentrations of GO/GA or different types of regulations.

An intracellular production of glyoxals is known to be enhanced by an oxidative stress (Abordo *et al.*, 1999). Thus, it appears that aldehyde removal is intimately associated with oxidative stress, e.g. GA-dependent induction of SoxR/S regulon (Benov and Fridovich, 2002). Oxidation of GA is one of the pathways generating GO (Okado-Matsumoto and Fridovich, 2000). Several enzymes have been implicated for metabolic removal of GA, e.g. AldA and FucO converting GA to glycolic acid or 1,2-ethandiol, respectively (Baldoma and Aguilar, 1987; Obradors *et al.*, 1998). From our study, it was concluded that although GA is toxic to cells, reduction of GO to GA, mediated by AKRs, is still critical in protecting cells from GO toxicity. We also found that MG and GO are preferably detoxified via different pathways, i.e. AKRs for GO detoxification rather than the GSH-dependent glyoxalases known for MG. Since these detoxification pathways require different reducing cofactors, NADPH versus GSH, depletion of the cofactors might result in different types of burdens in cellular metabolism and physiology. Unraveling the redox preferences of glyoxal removals may provide a clue to understand their exact detoxification mechanisms.

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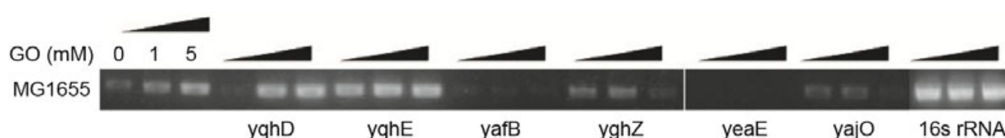

Fig. 3. Expression levels of AKRs upon GO treatment. To monitor the expression of AKR genes, qRT-PCR was performed using cDNA templates prepared from mRNAs of GO-treated cells (Lee *et al.*, 2010). The MG1655 strain was cultured to OD_{600} 1 in LB medium at 37°C with agitation. The cultures were then treated with 0, 1, and 5 mM of GO for 30 min, and their RNAs were isolated. Primers used are: for *yqhC*, yqhC-F (5′-AGATGAGCATTTCCAG CCGTCGAT-3′) and yqhC-R (5′-ATTTCGCGGATGATCTGTTTGCCG-3′); for *yqhD*, yqhD-F (5′-ACGCGAACAAATTCCTCACGATGC-3′) and yqhD-R (5′-GCTCAATACCGCCAAATTCCAGCA-3′); for *yqhE*, yqhE-F (5′-TCGAAGCATGGAAAGGCATGATCG-3′) and yqhE-R (5′-TGTTGCATC AGCGGATGAAGTTCG-3′); for *yafB*, yafB-F (5′-TGACGCGTGAGATCGGTATTTCCA-3′) and yafB-R (5′-ATATGGATGCCGTGCTGTTTAGCC-3′); for *yghZ*, yghZ-F (5′-TCAACGGCATTCCGCAAGATTCAC-3′) and yghZ-R (5′-CTTTCAGCAACCAGCTTAACGCCA-3′); for *yeaE*, yeaE-F (5′-TGGT ATATGGGCGAAGATGCCAGT-3′) and yeaE-R (5′-TTCTCTCGCAGACCGGTTAATGCT-3′); for *yajO*, yajO-F (5′-GTCGTTGCGACCAAAGTGTTC CAT-3′) and yajO-R (5′-TTCGATCGGCGTGTTGTAATCCCA-3′); for 16S rRNA, 16S rRNA-F (5′-CTGGTAGTCCACGCCGTAAA-3′) and 16S rRNA-R (5′-CGAATTAAACCACATGCTCCAC-3′). The qRT-PCR products were analyzed by agarose gel electrophoresis.

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