Chemical and Biological Engineering Publications   Chemical and Biological Engineering

2011

YqhD: A broad-substrate range aldehyde reductase with various applications in production of biorenewable fuels and chemicals

Laura R. Jarboe
Iowa State University, ljarboe@iastate.edu

YqhD: A broad-substrate range aldehyde reductase with various applications in production of biorenewable fuels and chemicals

Abstract

The Escherichia coli NADPH-dependent aldehyde reductase YqhD has contributed to a variety of metabolic engineering projects for production of biorenewable fuels and chemicals. As a scavenger of toxic aldehydes produced by lipid peroxidation, YqhD has reductase activity for a broad range of short-chain aldehydes, including butyraldehyde, glyceraldehyde, malondialdehyde, isobutyraldehyde, methylglyoxal, propanealdehyde, acrolein, furfural, glyoxal, 3-hydroxypropionaldehyde, glycolaldehyde, acetaldehyde, and acetol. This reductase activity has proven useful for the production of biorenewable fuels and chemicals, such as isobutanol and 1,3- and 1,2-propanediol; additional capability exists for production of 1-butanol, 1-propanol, and allyl alcohol. A drawback of this reductase activity is the diversion of valuable NADPH away from biosynthesis. This YqhD-mediated NADPH depletion provides sufficient burden to contribute to growth inhibition by furfural and 5-hydroxymethyl furfural, inhibitory contaminants of biomass hydrolysate. The structure of YqhD has been characterized, with identification of a Zn atom in the active site. Directed engineering efforts have improved utilization of 3-hydroxypropionaldehyde and NADPH. Most recently, two independent projects have demonstrated regulation of yqhD by YqhC, where YqhC appears to function as an aldehyde sensor.

Keywords

aldo-keto, glutathione, promiscuous, reverse engineering, tolerance

Disciplines

Biochemical and Biomolecular Engineering | Chemical Engineering | Petroleum Engineering

Comments

Laura R. Jarboe

YqhD: A broad-substrate range aldehyde reductase with various applications

in production of biorenewable fuels and chemicals

Department of Chemical and Biological Engineering

Iowa State University

Ames, IA 50011

515-294-2319 (phone), 515-294-2689 (fax), ljarboe@iastate.edu

Abstract

The *Escherichia coli* NADPH-dependent aldehyde reductase YqhD has contributed to a variety of metabolic engineering projects for production of biorenewable fuels and chemicals. As a scavenger of toxic aldehydes produced by lipid peroxidation, YqhD has reductase activity for a broad range of short-chain aldehydes, including butyraldehyde, glyceraldehyde, malondialdehyde, isobutyraldehyde, methylglyoxal, propanealdehyde, acrolein, furfural, glyoxal, 3-hydroxypropionaldehyde, glycolaldehyde, acetaldehyde and acetol. This reductase activity has proven useful for the production of biorenewable fuels and chemicals, such as isobutanol and 1,3- and 1,2-propanediol; additional capability exists for production of 1-butanol, 1-propanol and allyl alcohol. A drawback of this reductase activity is the diversion of valuable NADPH away from biosynthesis. This YqhD-mediated NADPH depletion provides sufficient burden to contribute to growth inhibition by furfural and 5-hydroxymethyl furfural, inhibitory contaminants of biomass hydrolysate. The structure of YqhD has been characterized, with identification of a Zn atom in the active site. Directed engineering efforts have improved utilization of 3-hydroxypropionaldehyde and NADPH. Most recently, two independent projects have demonstrated regulation of *yqhD* by YqhC, where YqhC appears to function as an aldehyde sensor.

Introduction

The production biorenewable chemicals at a high yield and titer, production of second-generation biofuels and mitigating biocatalyst inhibition by compounds in biomass hydrolysate are all current goals of the Metabolic Engineering and Synthetic Biology communities (Jarboe et al.). As we search metagenomic libraries for enzymes with novel functions and engineer existing enzymes for novel functions, it is easy to disregard the potential discovery of new, interesting enzymes in our workhorse biocatalysts. However, although it was virtually unknown in the scientific literature until 2003, the *E. coli* YqhD enzyme has proven vitally important in three recent projects: production of 1,3-propanediol (1,3-PD) and 1,2-propanediol (1,2-PD) at high yield and titer, production of the second-generation biofuel isobutanol and engineering of *E. coli* for furan tolerance. This enzyme has NADPH-dependent reductase activity for more than 10 aldehyde substrates (Table 1) and many of its products are valuable biorenewable fuels and chemicals.

Here, I review the use of this enzyme in various Metabolic Engineering projects as well as the current knowledge of its structure, regulation and kinetic parameters. This data is summarized in Tables 1 and 2 and Figure 1.

Propanediol production

YqhD came to the attention of the Metabolic Engineering community when researchers at duPont aimed to produce 1,3-PD using recombinant *E. coli* (Emptage *et al.* 2003), where 1,3-PD can be used as a monomer in the production of a variety of consumer products. These researchers used the *Klebsiella pneumonia* DHA regulon, but found that expression of *dhaT* resulted in accumulation of 3-hydroxypropionaldhyde (3-HPA) and other toxic aldehydes,

limiting biocatalyst viability. Removal of *dhaT* from the engineered pathway resulted in increased viability, as well as increased titer and yield 1,3-PD, motivating the search for 3-HPA reductase activity in the *E. coli* proteome. Fractionation of cell-free extracts and assessment of dehydrogenase activity using a native gel assay, according to the method of (Johnson and Lin 1987), yielded a single spot. Sequencing of this spot identified it as YqhD and subsequent deletion of *yqhD* confirmed that it is required for significant 1,3-PD production (Emptage et al. 2003).

Encouraged by these positive results, Wang and coworkers replaced *K. pneumonia*'s *dhaT* with *yqhD* in their recombinant *E. coli* strain, increasing 1,3-PD titer from 8.6 g/L to 13.2 g/L (Wang *et al.* 2007). Others have used YqhD in combination with the *Clostridium butyricum* DHA pathway in *E. coli*: co-expression of *yqhD* with *dhaB1* and *dhaB2* resulted in a strain that produced 104.4 g/L 1,3-PD from glycerol with a productivity of 2.61 g/L/hr (Tang *et al.* 2009). Mutagenesis of *E. coli* YqhD, described in more detail below, yielded a D99QN147H mutant with lowered K_m for 3-HPA; this mutant version produced twice as much 1,3-PD from 3-HPA by whole-cell transformation than the wild-type YqhD (Li *et al.* 2008).

K. pneumonia has the inherent ability to produce 1,3-PD from glycerol via DhaB and DhaT. However, like with Emptage's work with recombinant *E. coli*, 1,3-PD production was observed even after deletion of *dhaT* (Seo et al. 2010). A search of the *K. pneumonia* genome for a homolog of the *E. coli* YqhD found a match with 88% amino acid similarity. Increased expression of this homolog in a *ΔdhaD K. pneumonia* strain increased glycerol consumption and 1,3-PD yield. The final engineered strain produced 1,3-PD from glycerol at a titer of 7.7 g/L and yield of 0.438 g/g (Seo et al. 2010). Alternatively, Zhu *et al* expressed the *E. coli* YqhD in *K. pneumoniae* (Zhu *et al.* 2009). The strain had a slightly decreased growth rate relative to its

parent strain, but 1,3-PD titer, glycerol consumption and yield were all improved. The final engineered strain produced 67.6 g/L 1,3-PD from glycerol at a yield of 0.513 g 1,3-PD per g glycerol consumed.

Finally, the *E. coli* YqhD has contributed to 1,3-PD production in *Saccharomyces cerevisiae* (Rao *et al.* 2008). Through co-expression and chromosomal integration with the *K. pneumoniae dhaB*, the final engineered strain produced 1,3-PD at a titer of 0.4 g/L.

YqhD is also important for 1,2-PD production; 1,2-PD can be produced from acetol by D-aminopropanol dehydrogenase GldA, but a methylgoxyal reductase is required for production of acetol from methylglyoxal. A search for NAD(P)H-dependent methylglyoxyal reductase activity in anaerobic *E. coli* extracts found three candidates, all of which are NADPH-dependent: YqhD, YdhF and DkgA. A similar search in microaerobic cell fractions again identified YqhD and DkgA. The use of YqhD in subsequent metabolic engineering efforts resulted in production of 0.14 g 1,2-PD per g of glucose (Soucaille *et al.* 2008). YqhD was similarly important for production of 1,2-PD from glycerol by *E. coli* (Clomburg and Gonzalez 2010). Tuned expression of *gldA* and *mgsA* along with either *yqhD* or 1,2-propanediol oxidoreductase *fucO* showed that the *yqhD*-containing strain had a higher NADPH-dependent methylglyoxal reducing activity. A final *yqhD*-containing engineered strain produced 5.6 g/L 1,2-PD from glycerol with a mass yield of 0.213 g 1,2-PD per g of glycerol (Clomburg and Gonzalez 2010).

Thus, YqhD has played an invaluable role in production of two types of propanediol in three different biocatalysts. This is especially interesting when one considers that its K_m for 3-HPA is higher than most its other aldehyde substrates (Table 1, Figure 1).

Isobutanol production

Long-chain alcohols, such as butanol, have many desirable properties relative to existing biofuels such as ethanol (Atsumi *et al.* 2010). In an effort to produce isobutanol through a 2-keto-acid based pathway in *E. coli*, Atsumi *et al* needed an alcohol dehydrogenase to convert isobutyraldehyde to isobutanol (Atsumi et al. 2010). YqhD, due to its known activity as an aldehyde reductase, was a strong candidate for this desired activity. Subsequent deletion of *yqhD* decreased isobutanol production by 80%, confirming that it was responsible for the majority of isobutyraldehyde reductase activity. Comparison to ADH2 from *S. cerevisiae* and AdhA from *Lactobaccilus lactis* showed that YqhD and AdhA both outperform ADH2 in terms of isobutanol productivity. Attempts to increase YqhD activity by expressing YqhD from high-copy plasmids yielded no improvement in isobutanol production, indicating that basal expression of YqhD from the chromosome is sufficient for saturation of the isobutanol production pathway. YqhD was also used for industrial production of isobutanol by *E. coli* from glucose and sucrose (Donaldson *et al.* 2007).

Tolerance to Toxic Aldehydes

In the previous examples, YqhD was utilized for its metabolic activity, helping to convert an aldehyde intermediate to a valuable product. However, aldehydes also occur in non-metabolic pathways and YqhD contributes to reduction of these toxic compounds. Indeed, this job as an aldehyde scavenger may be the default role of YqhD in wild-type metabolism.

Lipid peroxidation by reactive oxygen species results in the production of short-chain aldehydes (Perez *et al.* 2008). YqhD has NADPH-dependent reductase activity towards many of these toxic aldehydes, such as acrolein, butyraldehyde and glyoxal (Table 1) (Perez et al. 2008; Lee et al. 2010). This activity plays a defensive role against reactive oxygen species-generating agents, such as hydrogen peroxide and potassium tellurite: deletion of *yqhD* increased growth

sensitivity and increased expression of YqhD decreased growth sensitivity (Perez et al. 2008). Similar results were observed for glyoxal and methylglyoxal: deletion of *yqhD* increased sensitivity and increased YqhD expression increased tolerance (Lee et al. 2010). Alternatively, sensitivity to Cd^{2+} or diamide, which do not generate reactive oxygen species, was not altered by changes in *yqhD* expression (Perez et al. 2008).

While the above examples demonstrate that reduction of toxic intermediates can be beneficial, this expenditure of valuable NADPH can also limit growth, as shown in Miller *et al*'s work with furfural. Lignocellulosic biomass is an attractive source of sugars for fermentation, but treatment of this biomass to release the fermentable sugars also releases inhibitory compounds, such as the aldehyde furfural. Reverse engineering of a furfural-tolerant mutant of ethanologenic *E. coli* W, via transcriptome and phenotype analysis, revealed that *yqhD* expression was decreased 50-fold and this transcriptional silencing was crucial to the tolerant phenotype (Miller et al. 2009b). Deletion of *yqhD* from the parent strain conferred furfural tolerance and restoration of *yqhD* expression in the evolved strain restored sensitivity. Decreased YqhD expression was accompanied by decreased furfural reduction and subsequent *in vitro* assays confirmed that YqhD is an NADPH-dependent furfural reductase. This link between furfural tolerance and YqhD-mediated furfural reduction was attributed to the finite availability of NADPH for biosynthesis (Miller et al. 2009b). Conversion of sulfate to sulfide, critical for cysteine biosynthesis, is NADPH-intensive; depletion of NADPH by YqhD with its low NADPH K_m (8 μM) leaves insufficient NADPH for cysteine biosynthesis (Miller et al. 2009a). YqhD is similarly important for tolerance to 5-hydroxymethyl furfural (Miller et al. 2010). As discussed below, the transcriptional silencing of *yqhD* in the furfural-tolerant mutant has been attributed to an insertion mutation within the regulator YqhC (Turner *et al.* 2010). In contrast to the

aldehydes generated by lipid peroxidation, the NADPH burden associated with furan reduction has more of an impact on growth than the toxic effect of the furans themselves.

Structure and Kinetic Parameters

Sequence analysis places YqhD in the so-called "iron-containing" alcohol dehydrogenase family, despite the fact that members of this group contain various divalent ions. Sulzenbacher *et al* performed a thorough characterization of YqhD's structure and kinetic properties (Sulzenbacher *et al.* 2004), with the conclusion that YqhD is an NADPH-dependent alcohol dehydrogenase that works best with alcohol chains longer than 3 carbons. However, as described above, recent analyses have suggested that aldehyde substrates are preferred relative to alcohols (Table 1, Table 3).

Each YqhD monomer contains an α-helical domain and a Rossmann-type fold domain. The 387-residue monomers assemble into a dimer, with dimer interactions including hydrogen bonding between the β-sheets of the coenzyme-binding domains and helices with residues 212-219 and 242-255 (Sulzenbacher et al. 2004). This dimer interaction is similar to the *Thermotoga maritima* ADH (Schwarzenbacher *et al.* 2004). However, unlike *T. maritama*'s ADH, YqhD's active site contains a Zn^{2+} atom instead of Fe^{2+} (Sulzenbacher et al. 2004). Sulzenbacher's studies showed that Asp194, His267 and His281 coordinate the zinc ion (Figure 2). Note that these sites are conserved in *Klebsiella* (Seo et al. 2010). The coenzyme binding is quite different from the classical Rossmann domain: NADP binds roughly perpendicularly to the parallel sheet of YqhD, instead of the parallel binding seen with horse liver ADH (Sulzenbacher et al. 2004). This means that the nicotinamide is nearly three times further away from the parallel sheet in YqhD than in the horse liver ADH. These structural studies noted the existence of a modified

NADP coenzyme, NADPH(OH)$_2$, but stated that this could be attributed to performing assays in non-physiological conditions (Sulzenbacher et al. 2004).

YqhD's NADP-dependent activity was queried with 11 alcohols, 15 aldehydes, 3 amino acids, 6 sugars and 4 α-OH acids. Significant activity was observed only for the alcohols 1-propanol, 1-butanol, 1-pentanol, isoamyl, 1-hexanol, 1-octanol and benzyl (Table 3). However, these seven substrates had relatively high K_m values, indicating that these alcohols are probably not the preferred substrates for these enzymes. In retrospect, it is now known that YqhD primarily functions as an NADPH-dependent aldehyde reductase, not the NADP-dependent alcohol dehydrogenase that was screened for in the above studies.

Directed Engineering

Error-prone PCR of YqhD in order to improve 1,3-PD production yielded two mutants, D99QN147H and Q202A, with decreased K_m and increased k_{cat} for certain aldehydes, particularly 3-HPA (Li et al. 2008) (Table 1). Note that the reported k_{cat} values for 3-HPA are lower than the normal range expected for this type of measurement. The D99QN147H mutant is consistent with the existing structural model (Sulzenbacher et al. 2004), as residues Asp99 and Asn147 both interact with NADPH. The benefit of mutation of Gln202 is unclear, as it has not previously been recognized as an important residue. As described above, use of the D99QN147H mutant increased 1,3-PD production from 3-HPA 2-fold (Li et al. 2008). Metabolic Explorer has also performed directed engineering and has isolated YqhD mutants with a 2-fold increase in the catalytic efficiency of YqhD toward NADPH (*Francois Voelker, personal communication*).

Regulation

While YqhD has been fairly well-characterized in terms of structure, kinetic parameters and substrate range, until recently its regulation was virtually unknown.

Three different projects involving whole-cell directed engineering showed perturbed expression of *yqhD*. As discussed above, evolution of an *E. coli* W derivative for furfural tolerance resulted in a nearly 50-fold decrease in *yqhD* transcript abundance (Miller et al. 2009b). Contrastingly, when Riehle *et al* evolved three parallel *E. coli* lines for thermotolerance, *yqhD* transcript abundance was increased approximately 4-fold in two of these lines relative to the parental *E. coli* B-derived strain (Riehle *et al.* 2003; Riehle *et al.* 2005). Finally, glyoxal-tolerant mutants of MG1655 were found to have increased expression of *yqhD* (Lee et al. 2010). This perturbed expression of *yqhD* was shown to be critical for furfural and glyoxal tolerance (Lee et al. 2010; Miller et al. 2009b), but the contribution of increased *yqhD* expression to thermotolerance has not been determined.

Reverse engineering efforts revealed that both the furfural-tolerant and glyoxal-tolerant strains contained a critical mutation within *yqhC*, a putative regulator that is adjacent to *yqhD*, though controlled by a separate promoter (Turner et al. 2010; Lee et al. 2010). The furfural-tolerant strain contained an insertion mutation, rendering YqhC unable to activate *yqhD* (Turner et al. 2010). The glyoxal-tolerant strain contained a P63S mutation that rendered YqhC constitutively active (Lee et al. 2010). Gel shift studies confirmed binding of YqhC to the *yqhD* promoter and identified the YqhC binding site (Lee et al. 2010). Subsequent experiments showed that not only is YqhC required for furfural-mediated activation of *yqhD*, but it is also required for activation of *yqhD* in response to other aldehydes, such as acetaldehyde, propionaldehyde, butyraldehyde, 5-hydroxymethyl furfural and cinnamaldehyde (Turner et al. 2010).

Transcriptome analysis of a *yqhC* deletion mutant, as well as the evolved strain that contains an insertion mutation in *yqhC*, led to the conclusion that YqhC is a localized regulator

and its regulon may consist solely of *yqhD* and *dkgA* (Turner et al. 2010). Bioinformatic analysis of other Gram-negative bacteria suggests that *yqhC* and *yqhD* have evolved in close association, with *dkgA* becoming associated more recently (Turner et al. 2010).

As described above, YqhD activity is important for reduction of the toxic aldehydes produced by lipid peroxidation (Perez et al. 2008). The finding that *yqhD* expression is increased in response to aldehyde-generating potassium tellurite is consistent with the proposed model that YqhC is activated by aldehydes. While a putative SoxS binding site was identified in the *yqhD* promoter, *yqhD* was still tellurite-sensitive in the absence of SoxS, leading Perez et al to conclude that induction of *yqhD* is largely independent of SoxS (Perez et al. 2008). This binding site has been recently attributed to the SoxS homolog YqhC (Lee et al. 2010).

Additional information about regulation of *yqhD* can be gleaned from the existing pool of *E. coli* omics data (University of Oklahoma Gene Expression Database 2009). Enterohemorragic *E. coli* had increased transcript abundance of *yqhD* in biofilm-associated cells treated with isatin relative to planktonic cells also treated with isatin (Lee *et al.* 2007). Isatin is an oxidized indole with two ketone groups and was being investigated due its role as a potential signaling molecule. There was no difference in *yqhD* transcript abundance between the planktonic and biofilm-associated cells that were not treated nor was there an expression difference in biofilm-associated and planktonic cells treated with 7-hydroxyindole. Note that 7-hydroxyindole does not have any aldehyde or ketone groups. This raises the intriguing possibility that isatin-mediated activation is due to the ketone groups, though it is unclear why *yqhD* is differentially expressed in the planktonic and biofilm-associated cells during isatin treatment.

Challenge of *E. coli* DH1 with n-butanol during mid-log growth transiently activated *yqhD*; 6-fold induction was observed at 30 minutes, with the 80 and 195 minute samples

showing less than a 2-fold change relative to the control sample (Rutherford et al. 2010).

Additionally, a BW25113 *yqhD* deletion mutant showed increased sensitivity to butanol

challenge (Rutherford et al. 2010). Given that superoxide dismutase *sodA* was also activated, the

authors attributed this *yqhD* activation to oxidative stress. This is consistent with the proposed

model that oxidative stress promotes lipid peroxidation, with the resulting aldehyde products

activating YqhC and *yqhD*. Deletion of *yqhD* could increase butanol sensitivity as a result of

accumulation of these toxic intermediates.

   yqhD was activated 4-fold following Cd^{2+} treatment of *E. coli* strain W3110 (Helbig *et al.*

2008). Cd^{2+} has a high affinity for sulfide, resulting in limitation of cysteine biosynthesis

(Helbig et al. 2008), similar to the effect seen following furfural challenge (Miller et al. 2009a).

This Cd^{2+}-mediated activation of *yqhD* was still observed in the absence of *gshA* or *gshB*, critical

components of the biosynthesis pathway for *E. coli*'s main thiol, glutathione (Helbig et al. 2008).

Glutathione is a critical component of the aldo-keto reductase system that converts potentially-

toxic aldehydes to alcohols (Perez et al. 2008). In addition to its thiol group, glutathione, like

isatin, contains two ketone groups. While the conservation of Cd^{2+}-mediated activation in the

absence of *gshA* or *gshB* suggests that *yqhD* regulation is not related to these two enzymes,

deletion of *gshA* or *gshB* resulted in increased abundance of *yqhD* relative to the wild-type, both

for untreated samples and for those treated with Cd^{2+}, suggesting that there is a link between

glutathione production and expression of *yqhD* (Helbig et al. 2008) Note that other studies found

that neither increased expression nor deletion of *yqhD* altered Cd^{2+} sensitivity in *E. coli*

BW25113, indicating that activation of *yqhD* does not significantly contribute to Cd^{2+} resistance

(Perez et al. 2008).

Finally, treatment with 1 mM S-nitrosoglutathione (GSNO) increased *yqhD* transcript abundance by 2-fold (Mukhopadhyay *et al.* 2004). Note that 0.1 mM GSNO did not perturb *yqhD* expression (Jarboe et al. 2008; Mukhopadhyay et al. 2004). GSNO has been shown to decrease cysteine bioavailability in *E. coli* (Jarboe et al. 2008), but here it seems more plausible that the ketone groups of S-nitrosoglutathione are sufficient for activation of YqhC. Alternatively, the Cd^{2+}, GSNO and furfural treatments all activate *yqhD* and cause limitation of cysteine biosynthesis, suggesting that there may be an as-yet unidentified link between *yqhD* and cysteine or sulfide biosynthesis pathways.

Unexplored functions

As shown in Table 1, YqhD has NADPH-dependent reductase activity towards at least twelve aldehydes and only a few of these have been explicitly recruited for Metabolic Engineering projects. However, it is plausible that YqhD has surreptitiously contributed to such projects. For example, YqhD has a K_m value of approximately 0.5 mM for butyraldehyde; this is lower than the reported 1.6 mM for *Clostridium acetobuylicum*'s butyraldehyde dehydrogenase (Palosaari and Rogers 1988) and 4.7 mM for aldehyde dehydrogenase ALDH in *Lactobaccilus brevis* (Berezina et al. 2010). Thus, it seems that the high background level of butyraldehdye reductase activity observed in *E. coli* by Inui *et al* could be attributed to YqhD (Inui *et al.* 2008). Similarly, *S. cerevisiae* ADH2 was used for production of 1-butanol and 1-propanol in *E. coli* (Shen and Liao 2008), but given YqhD's low K_m for butyraldehyde and propanaldehyde, it is reasonable to speculate that at least some of the observed activity was due to YqhD, as seen with the isobutanol production project by the same research group.

Other substrates appear to be truly untapped thus far. The reduction of acrolein to allyl alcohol is especially intriguing, given the current interest in selective hydrogenation of acrolein

to allyl alcohol in the chemical industry, where acrolein can be produced by selective

dehydration of glycerol (Claus 1998; Gallezot and Richard 1998; Liu et al. 2010). Acrolein can

be metabolically produced from 3-HPA by spontaneous thermal intramolecular dehydration, a

reaction that is enhanced at low pH (Schutz and Radler 1984). YqhD also has a low K_m for

malondialdehyde and could perform two reduction reactions on this substrate to produce 1,3-PD

(Table 1). However, there are few, if any, pathways other than lipid peroxidation that produce

malondialdehyde (Maness *et al.* 1999).

Discussion

While YqhD has a surprising number of substrates, the established degree of substrate

promiscuity pales in comparison to such enzymes as tyrocidine synthetase 1 (TycA), with a

documented 30 substrates (Babtie et al. 2010; Villiers and Hollfelder 2009). TycA's k_{cat}/K_m

values vary by 7 orders of magnitude; the available YqhD values vary by 6 orders of magnitude,

but exclusion of the extremely low k_{cat} value for 3-HPA leaves a range of 4 orders of magnitude

for YqhD. Pending an exhaustive search such as that performed by (Villiers and Hollfelder

2009), it is difficult to compare YqhD's substrate promiscuity to TycA. Other aldo-keto

reductases with a broad range of substrate specificity have been identified, including AKR from

Helicobacter pylori and YvgN and YtbE from *Bacillus subtilis*, though these enzymes show a

preference for nitrobenzaldehydes, chlorobenzaldehydes and pyridine aldehydes in contrast to

the relatively simple aldehydes reported to be utilized by YqhD (Cornally *et al.* 2008; Lei *et al.*

2009).

Regardless of if it meets the criteria of being a promiscuous enzyme, the involvement of

YqhD in so many distinct pathways makes it a very interesting enzyme. The fact that such a

useful, versatile enzyme remained in relative obscurity for many years despite the widespread

use of *E. coli* highlights the fact that there are other useful enzymes still to be discovered in our workhorse biocatalysts. Future characterization of the activation mechanism of YqhC and incorporation of YqhD into Metabolic Engineering projects will be useful for this intriguing system.

Acknowledgements

Abbreviations: 1,2-propanediol (1,2-PD), 1,3-propanediol (1,3-PD), 3-hydroxypropionaldhyde (3-HPA), alcohol dehydrogenase (ADH).

Table 1: NADPH-dependent aldehyde reduction by YqhD. k_{cat}/K_m values were calculated from the given values. For butyraldehyde, average K_m and average k_{cat} values were used. For acetaldehyde, two values were calculated using an average K_m value but each of the individual k_{cat} values. 5-hydroxymethylfurfural is not listed because no kinetic parameters have been reported. Substrates are sorted according to the average K_m value. Reactions utilized for metabolic engineering projects, as discussed in the text, are indicated with bold font.

[1]Perez et al. 2008

[2]Lee et al. 2010

[3]Atsumi et al. 2010

[4]Miller et al. 2009b

[5]Li et al. 2008

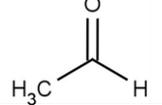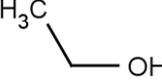| substrate, product	substrate structure	product structure	K_m (mM)	k_{cat} (s⁻¹)	k_{cat}/K_m (s⁻¹ mM⁻¹)	source
butyraldehyde, butanol			0.67, 0.40	60, 33	87	1,2
glyceraldehyde, glycerol			1.4	3.4	2.4	2
malondialdehyde, 3-HPA			1.8	60	33	1
isobutyraldehyde, isobutanol			2	1.0	0.5	3
methylglyoxal, acetol			2.6	4.7	1.8	2
propanaldehyde, propanol			3.3	45	14	1
acrolein, allyl alcohol			4.8	63	13	1
furfural, furfuryl alcohol			9	*n/a*	*n/a*	4
glyoxal, glycolaldehyde			12	10	0.83	2
3-HPA,: wt YqhD 1,3-PD			17	2.2×10^{-3}	1.3×10^{-4}	5
3-HPA: D99QN147H			8.0	5.0×10^{-3}	6.3×10^{-4}	5
3-HPA: Q202A			11	3.7×10^{-3}	3.4×10^{-4}	5
glycolaldehyde, ethylene glycol			28	54	1.9	2
acetaldehyde, ethanol			30, 29	1.1, 54	0.033, 1.8	1, 3
acetol, 1,2-PD			77	2.4	0.031	2
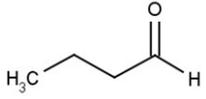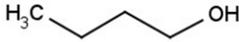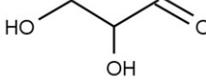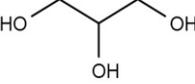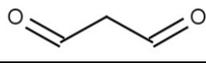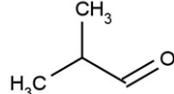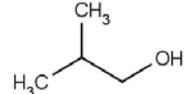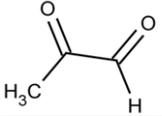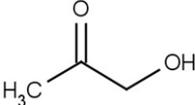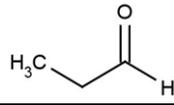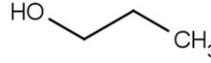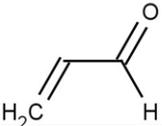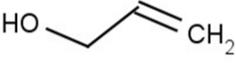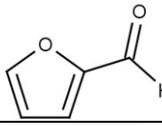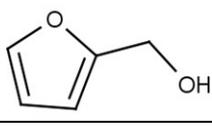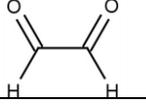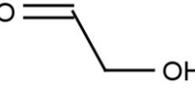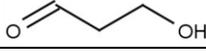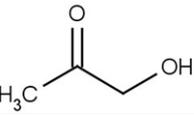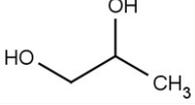
			0.008	*n/a*	*n/a*	4
NADPH						

Table 2: Biocatalysts engineered for the production of biorenewable compounds that incorporate YqhD.

Product	Organism, strain	Product Titer (g L^{-1})	Source
1,3-PD	*K. pneumonia*, derivative of Cu strain +pBR322-*orfWX-K. pneumonia yqhD*	7.5	(Seo et al. 2010)
1,3-PD	*K. pneumonia*, derivative of AK strain +pBR322-*orfWX-K. pneumonia yqhD*	4.7	(Seo et al. 2010)
1,3-PD	*K. pneumonia* ME-308 +pUC18-*E. coli yqhD*	67.6	(Zhu et al. 2009)
1,3-PD	*E. coli*, derivative of ER2925 +pBV220-*C. butyricum-dhaB-E. coli yqhD*	104.4	(Tang et al. 2009)
1,3-PD	*S. cerevisiae* W303-1A +pGAPZB-*K. pneumonia dhaB-E. coli yqhD*	0.40 ± 0.05	(Rao et al. 2008)
1,3-PD	*E. coli* derivative of BL21 +pET28a-*K.pneumonia dhaB –E. coli-yqhD*	13.2	(Wang et al. 2007)
1,2-PD	*E. coli* derivative of MG1655 +pTrcHis-*C. freundii dhaKL-E. coli gldA-mgsA-yqhD*	5.6	(Clomburg and Gonzalez 2010)
isobutanol	*E. coli*, derivative of BW25113	7	(Atsumi et al. 2010)

Table 3: K_m for NADP$^+$-dependent alcohol dehydrogenation by YqhD. Substrates are sorted according to the average K_m value. Values are the average $\pm$ one standard deviation, as reported in the indicated source.

substrate	K_m (mM)	source
benzyl alcohol	6 ± 2	(Sulzenbacher et al. 2004)
1-pentanol	9.0 ± 0.7	(Sulzenbacher et al. 2004)
1,3-propanediol	12.1	(Zhu et al. 2009)
1-hexanol	15 ± 2	(Sulzenbacher et al. 2004)
1-propanol	20 ± 10	(Sulzenbacher et al. 2004)
isoamyl alcohol	24 ± 3	(Sulzenbacher et al. 2004)
1-octanol	26 ± 8	(Sulzenbacher et al. 2004)
1-butanol	36 ± 4	(Sulzenbacher et al. 2004)
NADP$^+$	0.015-0.10, 0.15	(Zhu et al. 2009)

Figure 1: The role of YqhD in production of diverse biorenewable compounds. YqhD-mediated reactions are indicated in bold. Dashed lines indicate simplified pathway diagrams.

Figure 2: Interaction of the coenzyme and Zn with YqhD, as presented by and reproduced from (Sulzenbacher et al. 2004). Black represents carbon atoms, red represents oxygen atoms and blue represents nitrogen atoms. The dashed green lines indicate hydrogen bonds.

References

Atsumi S, Wu TY, Eckl EM, Hawkins SD, Buelter T, Liao JC (2010) Engineering the isobutanol biosynthetic pathway in *Escherichia coli* by comparison of three aldehyde reductase/alcohol dehydrogenase genes. Appl. Microbiol. Biotechnol. 85 (3):651-657.

Babtie A, Tokuriki N, Hollfelder F (2010) What makes an enzyme promiscuous? Curr. Opin. Chem. Biol. 14 (2):200-207.

Berezina OV, Zakharova NV, Brandt A, Yarotsky SV, Schwarz WH, Zverlov VV (2010) Reconstructing the *Clostridial* n-butanol metabolic pathway in *Lactobacillus brevis*. Appl. Microbiol. Biotechnol. 87 (2):635-646.

Claus P (1998) Selective hydrogenation of alpha,beta-unsaturated aldehydes and other C=O and C=C bonds containing compounds. Top. Catal. 5 (1-4):51-62

Clomburg J, Gonzalez R (2010) Metabolic engineering of *Escherichia coli* for the production of 1,2-propanediol from glycerol. Biotechnol. and Bioeng. (*submitted*)

Cornally D, Mee B, MacDonaill C, Tipton KF, Kelleher D, Windle HJ, Henehan GTM (2008) Aldo-keto reductase from *Helicobacter pylori* - role in adaptation to growth at acid pH. FEBS J. 275 (12):3041-3050.

Donaldson GK, Eliot AC, Flint D, Maggio-Hall LA, Nagarajan V (2007) Fermentative production of four carbon alcohols. WO 2007/050671 A2

Emptage M, Haynie SL, Laffend LA, Pucci JP, Whited G (2003) Process for the biological production of 1,3-propanediol with high titer. US 6514733 B1

Gallezot P, Richard D (1998) Selective hydrogenation of alpha,beta-unsaturated aldehydes. Catal. Rev. Sci. Eng. 40 (1-2):81-126

Helbig K, Grosse C, Nies DH (2008) Cadmium toxicity in glutathione mutants of *Escherichia coli*. J. Bacteriol. 190 (15):5439-5454.

Inui M, Suda M, Kimura S, Yasuda K, Suzuki H, Toda H, Yamamoto S, Okino S, Suzuki N, Yukawa H (2008) Expression of *Clostridium acetobutylicum* butanol synthetic genes in *Escherichia coli*. Appl. Microbiol. Biotechnol. 77 (6):1305-1316.

Jarboe LR, Hyduke DR, Tran LM, Chou KJY, Liao JC (2008) Determination of the *Escherichia coli* S-nitrosoglutathione response network using integrated biochemical and systems analysis. J. Biol. Chem. 283 (8):5148-5157.

Jarboe LR, Zhang XL, Wang X, Moore JC, Shanmugam KT, Ingram LO Metabolic engineering for production of biorenewable fuels and chemicals: Contributions of synthetic biology. J. Biomed. Biotechnol.

Johnson EA, Lin ECC (1987) *Klebsiella pneumoniae* 1,3-propanediol-nad+ oxidoreductase. J. Bacteriol. 169 (5):2050-2054

Lee C, Kim I, Lee J, Lee KL, Min B, Park C (2010) Transcriptional activation of the aldehyde reductase *yqhD* by YqhC and its implication in glyoxal metabolism of *Escherichia coli* K-12. J. Bacteriol. 192 (16):4205-4214

Lee J, Bansal T, Jayaraman A, Bentley WE, Wood TK (2007) Enterohemorrhagic *Escherichia coli* biofilms are inhibited by 7-hydroxyindole and stimulated by isatin. Appl. Environ. Microbiol. 73 (13):4100-4109.

Lei J, Zhou YF, Li LF, Su XD (2009) Structural and biochemical analyses of yvgN and ytbE from *Bacillus subtilis*. Protein Sci. 18 (8):1792-1800.

Li HM, Chen J, Li YH (2008) Enhanced activity of YqhD oxidoreductase in synthesis of 1,3-propanediol by error-prone PCR. Prog. Nat. Sci. 18 (12):1519-1524.

Liu Y, Tuysuz H, Jia CJ, Schwickardi M, Rinaldi R, Lu AH, Schmidt W, Schuth F (2010) From glycerol to allyl alcohol: Iron oxide catalyzed dehydration and consecutive hydrogen transfer. Chem. Commun. 46 (8):1238-1240.

Maness PC, Smolinski S, Blake DM, Huang Z, Wolfrum EJ, Jacoby WA (1999) Bactericidal activity of photocatalytic TiO2 reaction: Toward an understanding of its killing mechanism. Appl. Environ. Microbiol. 65 (9):4094-4098

Miller EN, Jarboe LR, Turner PC, Pharkya P, Yomano LP, York SW, Nunn D, Shanmugam KT, Ingram LO (2009a) Furfural inhibits growth by limiting sulfur assimilation in ethanologenic *Escherichia coli* strain LY180. Appl. Environ. Microbiol. 75 (19):6132-6141.

Miller EN, Jarboe LR, Yomano LP, York SW, Shanmugam KT, Ingram LO (2009b) Silencing of NADPH-dependent oxidoreductase genes (*yqhD* and *dkgA*) in furfural-resistant ethanologenic *Escherichia coli*. Appl. Environ. Microbiol. 75 (13):4315-4323.

Miller EN, Turner PC, Jarboe LR, Ingram LO (2010) Genetic changes that increase 5-hydroxymethyl furfural resistance in ethanol-producing *Escherichia coli* LY180. Biotechnol. Lett. 32 (5):661-667.

Mukhopadhyay P, Zheng M, Bedzyk LA, LaRossa RA, Storz G (2004) Prominent roles of the NorR and Fur regulators in the *Escherichia coli* transcriptional response to reactive nitrogen species. Proc. Natl. Acad. Sci. USA 101 (3):745-750

Palosaari NR, Rogers P (1988) Purification and properties of the inducible coenzyme a-linked butyraldehyde dehydrogenase from *Clostridium acetobutylicum*. J. Bacteriol. 170 (7):2971-2976

Perez JM, Arenas FA, Pradenas GA, Sandoval JM, Vasquez CC (2008) *Escherichia coli* YqhD exhibits aldehyde reductase activity and protects from the harmful effect of lipid peroxidation-derived aldehydes. J. Biol. Chem. 283 (12):7346-7353.

Rao Z, Ma Z, Shen W, Fang H, Zhuge J, Wang X (2008) Engineered *Saccharomyces cerevisiae* that produces 1,3-propanediol from D-glucose. J. Appl. Microbiol. 105 (6):1768-1776.

Riehle MM, Bennett AF, Lenski RE, Long AD (2003) Evolutionary changes in heat-inducible gene expression in lines of *Escherichia coli* adapted to high temperature. Physiol. Genomics 14 (1):47-58.

Riehle MM, Bennett AF, Long AD (2005) Changes in gene expression following high-temperature

adaptation in experimentally evolved populations of *E. coli*. Physiol. Biochem. Zool. 78 (3):299-

315

Rutherford BJ, Dahl RH, Price RE, Szmidt HL, Benke PI, Mukhopadhyay A, Keasling JD (2010)

Functional genomic study of exogenous n-butanol stress in *Escherichia coli*. Appl. Environ.

Microbiol. 76 (6):1935-1945.

Schutz H, Radler F (1984) Anaerobic reduction of glycerol to propanediol-1.3 by *Lactobacillus brevis*

and *Lactobacillus buchneri*. Syst. Appl. Microbiol. 5 (2):169-178

Schwarzenbacher R, von Delft F, Canaves JM, Brinen LS, Dai XP, Deacon AM, Elsliger MA, Eshaghi S,

Floyd R, Godzik A, Grittini C, Grzechnik SK, Guda C, Jaroszewski L, Karlak C, Klock HE,

Koesema E, Kovarik JS, Kreusch A, Kuhn P, Lesley SA, McMullan D, McPhillips TM, Miller

MA, Miller MD, Morse A, Moy K, Ouyang J, Page R, Robb A, Rodrigues K, Selby TL,

Spraggon G, Stevens RC, van den Bedem H, Velasquez J, Vincent J, Wang XH, West B, Wolf G,

Hodgson KO, Wooley J, Wilson IA (2004) Crystal structure of an iron-containing 1,3-

propanediol dehydrogenase (tm0920) from *Thermotoga maritima* at 1.3 angstrom resolution.

Proteins-Struct. Funct. Genet. 54 (1):174-177.

Seo JW, Seo MY, Oh BR, Heo SY, Baek JO, Rairakhwada D, Luo LH, Hong WK, Kim CH (2010)

Identification and utilization of a 1,3-propanediol oxidoreductase isoenzyme for production of

1,3-propanediol from glycerol in *Klebsiella pneumoniae*. Appl. Microbiol. Biotechnol. 85

(3):659-666.

Shen CR, Liao JC (2008) Metabolic engineering of *Escherichia coli* for 1-butanol and 1-propanol

production via the keto-acid pathways. Metab. Eng. 10 (6):312-320.

Soucaille P, Meynial-Salles I, Voelker F, Figge R (2008) Microorganisms and methods for production of

1,2-propanediol and acetol. WO 2008/116853 A1

Sulzenbacher G, Alvarez K, van den Heuvel RHH, Versluis C, Spinelli M, Campanacci V, Valencia C, Cambillau C, Eklund H, Tegoni M (2004) Crystal structure of *E. coli* alcohol dehydrogenase YqhD: Evidence of a covalently modified NADP coenzyme. J. Mol. Biol. 342 (2):489-502.

Tang XM, Tan YS, Zhu H, Zhao K, Shen W (2009) Microbial conversion of glycerol to 1,3-propanediol by an engineered strain of *Escherichia coli*. Appl. Environ. Microbiol. 75 (6):1628-1634.

Turner PC, Miller EN, Jarboe LR, Baggett CL, Shanmugam KT, Ingram LO (2010) YqhC regulates transcription of the adjacent *Escherichia coli* genes *yqhD* and *dkgA* that are involved in furfural tolerance. J. Ind. Microbiol. Biotechnol. *in press*

University of Oklahoma Gene Expression Database. (2009). http://genexpdb.ou.edu/, accessed July 24th 2010.

Villiers BRM, Hollfelder F (2009) Mapping the limits of substrate specificity of the adenylation domain of TycA. Chembiochem 10 (4):671-682.

Wang FH, Qu HJ, Zhang DW, Tian PF, Tan TW (2007) Production of 1,3-propanediol from glycerol by recombinant *E. coli* using incompatible plasmids system. Molec. Biotechnol. 37:112-119.

Zhu JG, Li S, Ji XJ, Huang H, Hu N (2009) Enhanced 1,3-propanediol production in recombinant *Klebsiella pneumoniae* carrying the gene *yqhD* encoding 1,3-propanediol oxidoreductase isoenzyme. World J. Microbiol. Biotechnol. 25 (7):1217-1223.