Bacterial group III alcohol dehydrogenases-function, evolution and biotechnological applications

S Elleuche*, G Antranikian

Abstract

Introduction
Living organisms have developed strategies to cope with alcoholic substrates. Due to the manifold appearance of alcoholic compounds, enzymes that are capable of decomposing or processing such molecules have emerged several times during evolution. These proteins are the so-called alcohol dehydrogenases, a group of ubiquitous enzymes that catalyse the interconversion between alcohols and aldehydes or ketones. In addition to several other co-factors that are used by alcohol dehydrogenases, three non-homologous protein families exclusively use NAD(P)/NAD(P)H. Two of the three protein families have been predominantly and extensively studied in animals; this short review focuses on bacterial group III alcohol dehydrogenases and sheds some light on their functionality, distribution and potential biotechnological applications.

Conclusion
In contrast to other NAD(P)-dependent ADHs, there is only little information on group III ADHs; however, recent investigations have shown some promise for some of these enzymes. The continuous evaluation of such biocatalysts will aid in the development of environment friendly approaches for the production of fine chemicals, which could provide an alternative to traditional petrochemical routes.

Introduction
Alcohol dehydrogenases (ADHs; EC 1.1.1.1 and EC 1.1.1.2), an omnipresent group of enzymes, catalyse the interconversion between alcoholic compounds and their corresponding aldehydes or ketones, an activity essential for different processes in living organisms. These enzymes are abundant in animals, plants, fungi, algae, bacteria and the Archaea domain. They are present in diverse tissues, such as the liver, kidney, gastric mucosa and mammary glands, as well as in microorganisms thriving in extreme environments, such as solfataric fields or hot vents. To catalyse the reduction of aldehydes or oxidation of alcohols, ADHs are in need of a co-factor which is used as an electron acceptor. Based on the utilisation of a specific co-factor, it is possible to group these proteins into three different categories: (1) nicotinamide adenine dinucleotide- (NAD-) or nicotinamide adenine dinucleotide phosphate- (NADP-) dependent ADHs, (2) pyrroloquinolinequinone-, haem- and (3) flavin adenine dinucleotide-dependent isozymes.

So far, there are three distantly related types of ADHs, which can be grouped as NAD(P)-dependent ADHs. The zinc-containing, long/medium chain ADHs (group I) are the most studied and can be distinguished from the superfamilies of the metal-free, short chain ADHs (group II) and the metal-containing ADHs (group III). Several organisms such as mosquitoes and flies have been described to contain ADHs of all the three different types. In contrast, the human genome comprises of at least seven genes encoding group I ADHs, and a single open reading frame which codes for an ADH belonging to group III. Furthermore, the mammalian enzyme horse liver alcohol dehydrogenase is a prototype of the first group, while a group II isozyme was first studied from the fruit fly Drosophila melanogaster. The third group of ADHs was first discovered in micro-organisms, and the first isozyme that has been studied in detail is a microbial ADH from the ethanol producing bacterium Zymomonas mobilis. In this review, we have briefly focused on the function, evolution and possible applications of the highly diverse group III of ADHs.

Discussion
The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institutions in which these studies were performed.

Function of group III ADHs
ADHs fulfil multiple physiological roles and display a wide variety of substrate specificities by exhibiting great substrate promiscuity. However, not every isozyme is capable of oxidising short chain aliphatic compounds, such as ethanol. This can also be illustrated by the transcriptional expression profile of ADH encoding genes. While the expression of group II ADH genes is usually induced by ethanol exposure, this is not true for other NAD(P)-dependent ADHs. They often show low affinity and catalytic efficiency towards ethanol as a substrate, indicating again that these enzymes mainly resume versatile roles in the cell without participating in ethanol metabolism.

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metabolism\(^1\). Nevertheless, there is a reaction mechanism that is common for all NAD(P)-dependent ADHs: the reduction of an aldehyde or ketone results in the production of an alcoholic compound and an oxidised co-factor. The regeneration of NAD(P) is often essential for other coupled physiological reactions, especially in microorganisms. In agreement with this reaction, the oxidation of an alcohol results in the production of an aldehyde or ketone and supplies reduced NAD(P)H (Figure 1).

ADHs are capable of processing a wide variety of substrates including linear, branched, primary, secondary and circular alcohols in addition to their corresponding aldehydes and ketones. Within the third group of NAD(P)-dependent ADHs, some members are described and characterised as lactaldehyde dehydrogenases, butanol dehydrogenases, 1,3-propanediol (1,3-PD) dehydrogenases, while other isozymes exclusively catalyse the reduction of aldehydes without oxidising alcohols at all\(^9-12\). Therefore, it has been speculated, that some group III ADHs are mainly involved in aldehyde reduction in bacterial species rather than alcohol turnover.

The prototype enzyme YqhD from Escherichia coli, shows a rather high affinity to aldehydes coupled with low alcohol oxidation activity, which is in good agreement with group III ADHs from other microorganisms\(^8,12,13\). Due to the substrate looseness of group III ADHs, detailed characterisation of various candidate genes is necessary to fully understand their function-evolution relationships. For this reason, it is often hard to disentangle the physiological functions of these enzymes. Isozymes that have mainly been characterised so far include those from the family of Enterobacteriaceae, such as E. coli and Klebsiella pneumoniae and from the hemiascomycetous yeast Saccharomyces cerevisiae. Members from extremophilic micro-organisms, such as (hyper-) thermophilic archaeal and bacterial species, have also been investigated in detail\(^2,3,9,14,15\). Distantly related members of group III ADHs were encoded as pairs in enterobacterial species and characterised in detail. The enzyme FucO from E. coli is a lactaldehyde: propanediol oxidoreductase catalysing the NADH-dependent reduction of lactaldehyde to produce 1,2-propanediol (1,2-PD)\(^10,14\). The closely related protein DhaT from K. pneumoniae has been shown to be capable of converting 3-hydroxypropionaldehyde into 1,3-PD, which is important for NAD+-production under physiological conditions. Adh3.1 from the plant pathogenic enterobacterium Dickeya zeae, showed highest activity on short chain primary alcohols, but was unable to act on 1,2-PD or 1,3-PD\(^9,13\). These members are clustered together and named as lactaldehydes: propanediol oxidoreductase-like enzymes or named on

**Figure 1:** Reaction mechanism of alcohol dehydrogenases. These enzymes catalyse the NAD(P)-dependent interconversion of aldehydes or ketones and enantiomerically pure alcoholic compounds. The complete structure of NAD\(^+\)/NADH is indicated by a red H-atom, while the additional phosphate-group of NADPH is indicated in green.
the basis of the most studied prototype as DhaT-like enzymes. They can be opposing to the YqhD-like enzymes within the group III ADHs, which are mostly annotated as butanol dehydrogenases. Both groups show functionally overlapping as well as diverse properties (Table 1). Instead of NADH, YqhD-like enzymes exclusively use NADPH as a co-factor and prefer to catalyse the reduction of aldehydes to the oxidation of alcohols. This enzyme is of industrial importance due to its ability to produce 1,3-PD (see below). YqhD from *E. coli* and Adh3.2 from *D. zeae* are useful in detoxifying glyoxals13,16. It is hypothesised that *E. coli* YqhD might be involved in protecting the cells from the toxic effects of lipid-oxidation derived aldehydes. Additionally, the physiological role of DhaT from *K. pneumoniae* is speculated to be the regeneration of NAD⁺, which is needed as a co-factor in the oxidative glycerol degradation pathway12,17.

**Evolution of group III ADHs**

Enzyme evolution is mainly determined by functional convergence and functional diversification18. The fact that different non-homologous ADHs are capable of catalysing the interconversion of aldehydes and alcohols, although they are structurally distinct, makes these groups of enzymes an excellent candidate for functional convergent evolution. However, the ability to act on different substrates and/or to be involved in different metabolic pathways of orthologous ADHs within a specific group, may be the result of gene duplications and/or functional diversification.

Group III ADHs are not related to other NAD(P)-dependent ADHs, which means that they arose independently during the course of evolution. The complex and long evolutionary history of this group of enzymes reflects its physiological versatility. While only a single mitochondrial group III ADH has been identified in animal genomes, there have been several enzymes found in prokaryotes, executing multiple physiological roles and exhibiting diverse substrate specificities1,13. These enzyme variants might be the result of ancient gene duplications and functional diversification events. Gene duplications followed by functional innovation have been shown to appear very often in enzymes13,19-21. As a result of functional diversification within the third group of ADHs, these enzymes can be further subdivided and show substantially distinct substrate specificities and functions10,11,13-15,22. Moreover, it has been hypothesised that group III ADHs were distributed throughout the horizontal gene transfer between the Archaea and Bacteria domains, which might have involved functional diversification and non-orthologous gene displacement3.

The third group of ADHs contains enzymes that exhibit a size of around 385 amino acid residues (Figure 2). Pairs of the functionally characterised DhaT-like and YqhD-like members of the group III ADHs, show a low identity of approximately 20% over the complete length of the proteins, but show an overall structural similarity. Pairs of these enzymes were found and characterised in several bacterial species, such as *K. pneumoniae*, *E. coli* or *D. zeae*10,13-15,23,24. However, this was not the case in all of the members of the Enterobacteriaceae family. It was shown that another species of the genus *Klebsiella*, namely *K. oxytoca*, does not encode a DhaT-like ADH, but a protein that is specific to the basis of the most studied prototype as DhaT-like enzymes. They can be opposed to the YqhD-like enzymes within the group III ADHs, which are mostly annotated as butanol dehydrogenases. Both groups show functionally overlapping as well as diverse properties (Table 1). Instead of NADH, YqhD-like enzymes exclusively use NADPH as a co-factor and prefer to catalyse the reduction of aldehydes to the oxidation of alcohols. This enzyme is of industrial importance due to its ability to produce 1,3-PD (see below). YqhD from *E. coli* and Adh3.2 from *D. zeae* are useful in detoxifying glyoxals13,16. It is hypothesised that *E. coli* YqhD might be involved in protecting the cells from the toxic effects of lipid-oxidation derived aldehydes. Additionally, the physiological role of DhaT from *K. pneumoniae* is speculated to be the regeneration of NAD⁺, which is needed as a co-factor in the oxidative glycerol degradation pathway12,17.

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**Table 1. Substrate and cofactor specificities of group III ADHs.**

<table>
<thead>
<tr>
<th>Aldehydes</th>
<th>DhaT-like</th>
<th>YqhD-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-factor</td>
<td>NAD(H)</td>
<td>NADPH</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Propanol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Butanal</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Methanol</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glycerol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,2-PD</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>1,3-PD</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Alcohols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Propanal</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Butanal</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>3-HPA</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>Methyglyoxal</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>Glycoaldehyde</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; n.d., not determined; 1,2-PD, 1,2-propanediol; 1,3-PD, 1,3-propanediol; 3-HPA, 3-hydroxypropionaldehyde.
>80% identical to the YqhD enzyme from *E. coli*. Interestingly, this enzyme, YqhD-1, seems to compensate for the loss or the non-existence of the DhaT-like ADH by exhibiting low activity towards several alcoholic compounds. Although metal-ion containing ADHs appear alike in their tertiary structure, they differ in their quaternary structure. Dimeric, tetrameric, octameric and decameric states have been described in this superfamily. Moreover, there is a difference in the presence of the metal-ion in the conserved catalytic region of the group III ADHs. While iron or zinc were predominantly identified as catalytic ions, the recently solved structure of Adh3 from the gram-positive bacterium *Oenococcus oeni*, revealed the presence of a nickel ion in the dimeric structure of this protein. Another striking difference was found within the primary structure of the order Lactobacillales (including *O. oeni*), where the coordinating amino acid residues are asparagine, two histidines and an atypical glutamine which is replaced by a third histidine in well-characterised structures from other members of the group III ADHs (Figure 2). In addition, the metal ions of DhaT-like members of the group III ADHs were bound more tightly in comparison to the YqhD-like isozymes. Nevertheless, the great diversity within this group of enzymes complicates the functional annotation of newly identified sequences that are neither expressed nor characterised in detail.

Applications of group III ADHs

The history of the biotechnological applications of ADHs commenced more than 100 years ago when Nobel prize laureate Eduard Buchner established the process of alcohol fermentation without the utilisation of yeast cells. The history of this enzyme is also closely linked to the production of chemical compounds (e.g. 1,3-PD), as the application of DhaT and YqhD in recombinant *E. coli* small molecule can be used as a monomer for the production of a variety of products, such as synthetic polymers, drugs, fibres or cosmetics. A lot of effort has been made to generate multi-mutated, engineered, expression strains to increase the titre of 1,3-PD produced from glycerol. Moreover, it has been shown that ADHs YqhD and FucO are important for the production of 1,2-PD from glycerol in *E. coli* with YqhD exhibiting the additional advantage of reducing methylglyoxal to acetol; the latter compound has the ability of being converted to 1,2-PD by another enzyme, namely the D-aminopropanol dehydrogenase GldA.

As mentioned above, the physiological role of YqhD might be to act as an aldehyde scavenger, which...
prompted Lee and Park\textsuperscript{29} to develop another interesting application of YqhD. In a recent publication, these authors came up with a suicidal vector-cloning system which is based on the toxic effects of butanal and propanal in the host \textit{E. coli}\textsuperscript{29}. It has been shown that \textit{E. coli} is sensitive to butanal and other aldehydes, when \textit{yqhD}-encoding genes are overexpressed in a recombinant form and these aldehydes are reduced to give alcoholic compounds\textsuperscript{13,16,29}. In the presented system, the \textit{lacZ}-gene in vector pUC19 is replaced by a \textit{yqhD}-variant with a preceding multiple cloning site (MCS). The expression of \textit{yqhD} leads to lethality when \textit{E. coli} transforms are incubated on butanal- or propanal-containing plates. The insertion of a foreign DNA-fragment into the MCS interferes with the expression of \textit{yqhD}, which results in transformants that survive on aldehyde-containing growth plates\textsuperscript{29}.

**Conclusion**

ADHs are a family of proteins with a complex and interesting evolutionary history. This has led to the generation of enzymes with broad substrate promiscuity. These proteins can be involved in either a variety of distinct pathways or an exclusive catalysis of well-balanced specific reactions, often producing enantiomerically pure products. In contrast to other NAD(P)-dependent ADHs, there is only little information on group III ADHs so far. However, some recent investigations have proven the promising applicability of some of these enzymes. The continuous evaluation of such biocatalysts will open new avenues for environmentally friendly production of fine chemicals, which can effectively compete with traditional petrochemical routes. More-