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Full Length Research Paper

Investigating structure-activity relationships within *Enterococcus faecalis* 2-deoxyribose aldolase

Nathan Wymer*, Jeremy Steflik and Maria Brown

Pfizer Global Research and Development, Groton, Connecticut (CT), USA.

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The 2-deoxyribose aldolase (DERA) is an enzyme used for the synthesis and decomposition of carbohydrates. The DERA has been shown in previous studies to be utilized in an alternative metabolic pathway during stress. This article uses the *Enterococcus faecalis* DERA as a model system to study the structure-function relationship of amino acids towards DERA activity in hopes of garnering a better understanding of catalytic activity and possible mutations outside the binding pocket that might affect this activity. The active site was probed using saturation mutagenesis and identified two positions that increased activity (His187, Ser206). The outer shell amino acids were selected using bioinformatics. Mutagenesis of these outer shell amino acids combined with statistical analysis identified whether mutation of amino acids were advantageous or deleterious towards overall DERA activity without the need for high-throughput screening. These results will provide a basis to further study the intricate structure-activity relationships of amino acids within this DERA.

Key words: *Enterococcus faecalis*, 2-deoxyribose aldolase, structure-function relationships.

INTRODUCTION

The 2-deoxyribose aldolase (DERA) is a ubiquitous enzyme used in the synthesis and decomposition of carbohydrates, particularly nucleic acids (Wang et al., 1990; Heine et al., 2004; Liu, et al., 2004; Tao and Xu, 2009). The DERA catalyzes the reversible cleavage of 2-deoxyribose into glyceraldehyde-3-phosphate (G3P) and acetaldehyde, before it gets to metabolism. Both prokaryotic and eukaryotic cells can use nucleic acid degradation during metabolic stress to slow the progression of cell damage (Sgarrella et al., 1997). As such, the DERA may be a target to combat pathogenic bacteria metabolism. The *Enterococcus faecalis* DERA was used as a model system to better understand the DERA enzyme and to understand the protein structure-function relationships that drive DERA catalytic activity.

E. faecalis was chosen as a model system, because it is a common Gram-positive bacterium found in the gastrointestinal tracts of humans and other mammals. *Enterococcus* infections are well known in patients with prolonged hospital stays and undergoing multiple courses of antibiotics (Huycke et al., 1998; Murray, 2000). Like other pathogenic bacteria, *Enterococcus* bacteria have become resistant to a variety of antibiotics, including vancomycin (Huycke et al., 1998; Murray, 2000).

This study intends to understand one aspect of the metabolism of pathogenic bacteria in order to combat this increasing threat.

To better understand the structure-function relationships within the DERA, this study used bioinformatics and structure-guided selection methodology to identify amino acid positions that might negatively affect DERA catalytic activities. With a better understanding of the protein structure-function relationships of this DERA, this study can increase our knowledge of the precise catalytic and binding functions of amino acids within the active site as well as possible mutations that could provide resistance in the future.

*Corresponding author. E-mail: nathan.wymer@pfizer.com.

Abbreviations: DERA, 2-Deoxyribose aldolase; **G3P,** glyceraldehyde-3-phosphate; **TB,** terrific broth.

MATERIALS AND METHODS

Selection of mutations

Mutations outside the active site were selected using methods

Table 1. List of the mutations within DERA investigated during this study. The list contains both mutations selected using bioinformatics and structure-guided techniques as well as their overall impact on catalytic activity.

Bioinformatics selected mutations				Active site mutations			
Negative	**Neutral**		**Positive**	**Negative**		**Neutral**	**Positive**
I11L	I11V	Y75F	I24L	T10A	S154C	H187A	H187N
V21I	E15D	I81V	S35A	T10R	S154Q	H187R	H187C
F34Y	Q22E	I94V	Y104L	T10C	S154G	H187E	H187Q
F34A	I24V	S99A	S158A	T10Q	S154I	H187L	H187G
N86D	I25V	Q100E	R171K	T10G	S154L	H187K	H187M
I92L	D26E	Q100K	V186I	T10L	S154K	H187S	H187F
I92V	K29R	Q100G	I196V	T10P	S154M	S206G	H187T
Y102F	I38V	Q101D		T10S	S154P	S206M	H187W
Y104F	W42Y	Q101E		T10W	S154T	S206T	H187V
R106K	F45L	Q107E		H187L	S154W		S206A
D177E	S47A	Q110E		H187P	S154V		S206N
E191D	Q49A	G111A		S154A	S206R		S206C
A205T	Q49K	K119R		S206K	S206K		S206H
T207S	Q49E	V137I		S206F	S206F		S206I
T207A	D52G	K138R		S206W	S206W		S206V
V209I	D52E	I167V		S206Y	S206Y		
	T53S	R168K		S154D			
	I61V	M178L		S154C			
	E71A			S154Q			

previously described (Jonsson et al., 1993; Fox et al., 2003; Gustafsson et al., 2003). This selection process uses protein sequence alignments and bioinformatics to identify positions that are not conserved in a consensus sequence thus suggesting mutations that might not negatively impact activity. Sixty potential mutations were identified (Table 1). These mutations were then combined in a statistically significant variant set to provide relevant information into a machine-learning algorithm (Jonsson et al., 1993; Fox et al., 2003).

For the selection of positions within the active site, amino acids were identified using protein sequence alignments and relevant DERA crystal structures (Heine et al., 2004; Sakuraba et al., 2007). Four sites were hypothesized to hydrogen-bond to a G3P molecule (Table 1). These four sites were then subjected to saturation mutagenesis.

Gene synthesis and manipulation

The gene for the *E. faecalis* DERA enzyme (NCBI: NP_813976) was codon optimized for expression in *Escherichia coli* and then synthesized. For the statistical-based mutation screening, each of the DERA variants was synthesized *de novo* (DNA2.0, Menlo Park, CA). For the saturation mutagenesis of the active site amino acids, the mutations were prepared using the Quick-Change protocol (Stratagene, Santa Clara, CA). A degenerate NNK codon was used within the quick-change primers (IDT, Coralville, IA) to simultaneously prepare mutations at a specific site. Ninety six colonies were picked for each mutant site. These colonies were added to 1 ml TB broth (MoBio, Carlsbad, CA) containing antibiotic in a 2 ml microplate (VWR, Radnor, PA) and incubated overnight with shaking (37°C, 210RPM). The cells were then pelleted (20 min, 3000 xg's), and the medium was decanted. The plasmid DNA was extracted using miniprep technologies (Qiagen, Hilden, Germany) and sent for DNA sequencing. Usually, the full complement of saturation mutants was not completed from this initial 96 member library. Those missing mutants were prepared individually using new quick-change primers with appropriate codon changes.

DERA expression and activity

The DERA gene was cloned into a pSTRC18 plasmid. The pSTRC18 plasmid is a proprietary expression plasmid containing a modified inducible *trc* promoter and aaDA (streptomycin) antibiotic resistance gene. The DERA enzymes were expressed in BL21 (DE3) Gold cells (Stratagene, Santa Clara, CA). Single colonies were picked into 2 ml LB medium containing streptomycin (50 mg/L) and incubated overnight with shaking (37°C, 210RPM). After overnight incubation, 1 ml of seed culture was added into 50 ml autoinduction TB medium (Invitrogen, Carlsbad, CA) containing streptomycin (50 mg/L) in a 250 ml baffled-flask. The culture was then incubated overnight with shaking (30°C, 210RPM). The DERA enzyme expression was monitored with 4-20% Tris-Glycine SDS-PAGE (Invitrogen, Carlsbad, CA) and staining with coomassie. DERA activity was measured using methods similar to previously described (Wang et al., 1990; Heine et al., 2004; Liu et al., 2004; Sakuraba et al., 2007; Tao and Xu, 2009).

Statistical analysis of mutations

The activity contributions of the statistically prepared variant set were analyzed using machine learning by methods previously described (Jonsson et al., 1993; Fox et al., 2003).

Molecular structure model of DERA

A molecular structure model for the DERA was created using the *Thermotoga maritima* DERA crystal structure (100Y) as a template. (Sakuraba et al., 2007) The model was minimized using the in-house software package MoVIT (Figure 1). The DERA model shows a classical $\alpha\beta$-barrel fold with the catalytic amino acids in the center. As can be deduced by other DERA structural studies, K152 forms the imine intermediate with the acetaldehyde, D89 is the catalytic base, and K181 assists in modulating the pKa of the K152 side-chain amine (Heine et al., 2004; Sakuraba et al., 2007).

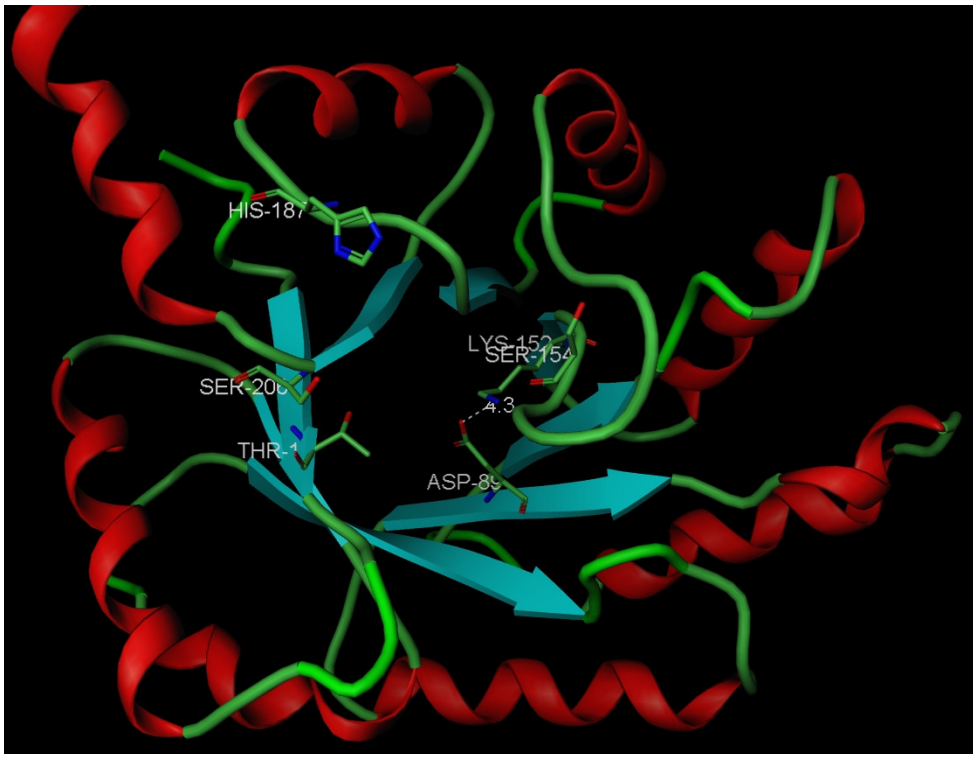

Figure 1. Molecular model of *E. faecalis* DERA. The structure has a classic αβ-barrel fold. The catalytic (D89, K152, K181) and binding pocket (T10, S154, H187, S206) amino acids are highlighted.

RESULTS AND DISCUSSION

Statistical analysis of the DERA activity assay shows that 16 of the 60 mutations chosen by bioinformatics had a clear negative influence on product formation (Table 1). Interestingly, several of these deleterious mutations occur at the same position (F34, I92, T207). These findings suggest how critical these positions can be to catalytic activity despite the original bioinformatics data suggesting these amino acid positions could accept mutation. Explaining the reasoning for their deleterious effects can be difficult, at best. The molecular modeling structure shows the F34 position helps form the rear cap of the active site. Modifying this amino acid could disrupt the catalytic residue K181 thus affecting the pKa of the imine-forming amino acid K152. Mutations at I92 and T207 are clearly detrimental, but like most of the deleterious mutations, they occur outside the active site. The exact reasons for these negative affects most likely occur from the disruption of the hydrogen bonding network of the active site, but more study is needed to determine the exact relationships.

The amino acids positions within the active site chosen for saturation mutagenesis are part of the hydrogen-bonding network for the G3P substrate (Heine et al., 2004; Sakuraba et al., 2007).

These residues help to stabilize the phosphate group as well as hydrogen bond to the C2-hydroxyl. All mutations at T10 and S154 gave mutant DERAs with lower catalytic activities than the WT. The H187 and S206 mutations also gave wider variety of changes in activities. Interestingly, the mutation H187R did not improve activity. Alignments of DERA protein sequences as well as crystal structures usually show lysine or arginine amino acids at this position, presumably to form an ionic interaction with the phosphate group of G3P. Simply adding a positive charge into the active site is not enough, thus the steric consequences of adding the larger arginine group within the active site must overcome the added benefits of this new ionic interaction.

Understanding which mutations increase activity can also be of importance. These mutations could provide a mechanism for resistance to inhibition while maintaining overall DERA activity. We are also currently investigating how these mutations individually or in combination could increase activity.

Conclusions

This study looked at ways to disrupt the catalytic activity of the *E. faecalis* DERA enzyme. New structure-function relationships can be identified within the DERA by combining bioinformatics and structure-guided approaches

to search for amino acids that have both positive and negative impacts on activity. These findings suggest that the most efficient method to disrupt catalytic activity is to disrupt the hydrogen-bonding network of the G3P substrate. Thus, the hydrogen-bonding network of the DERAs might be investigated further since mutation would most likely lead to significantly decreased DERA activity. This initial study helps to better understand the DERA as part of the overall carbohydrate metabolism of pathogenic bacteria and could lead to a new generation of antibiotics in the future.

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