The Structure and Function of Esterases from Lactic Acid Bacteria

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Abstract

Compounds derived from the breakdown of glyceride esters of milk fat, such as free fatty acids and short chain esters, are recognised as playing an important role in the flavour of a range of fermented foods. Esterases, capable of hydrolysing ester bonds, and in some cases, synthesising them *via* an acyltransferase mechanism, typically enter the fermentation from the starter and adjunct lactic acid bacteria that are used to inoculate milk to initiate the fermentation process. With such an important role in the development of both desirable and undesirable flavours, understanding how these enzymes operate is essential for product control. In this study, the crystal structures of three lactic acid bacterial esterases were solved: EstA from *Lactococcus lactis*, and AA7 from *Lactobacillus rhamnosus* which are both capable of hydrolysis of short chain triglycerides as well as synthesising esters *via* a transferase mechanism, and AZ4, an esterase from *L. rhamnosus* which appears to be limited to hydrolysis reactions. Whilst all three were found to be members of the αβ hydrolase family, unique features were found for each enzyme, reflecting the large differences in their primary sequences, substrate specificities and activities.

EstA and AA7 were both found to have a shallow substrate binding cleft, bisected by the catalytic machinery. The divided binding cleft suggests that during a transferase reaction the transferred group binds in one pocket, with the donor and acceptor groups (dependant on the stage of catalysis) binding in the other.

In contrast, AZ4 was found to have a single deep substrate binding cavity, extending into the enzyme interior, with the catalytic residues located near its entrance. The absence of a second binding site for an acceptor is consistent with AZ4 having only one function – that of a hydrolase.

The structures presented in this study are the first three dimensional structures of esterases from lactic acid bacteria to be reported. Their analyses, both in native form, and complexed with a varity of ligands mimicking various stages of the reaction cycle have highlighted how this basic fold can be adapted to efficiently catalyse different reactions. More importantly, in the case of AZ4, these structures have suggested that there is a novel mechanism used by the esterases to promote the enzyme reaction to proceed to completion, by preventing a futile catalytic reaction.

Enzymes are things invented by biologists that explain things which otherwise require harder thinking.

Jerome Lettvin

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Matthew Bennett 24 October 2007

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Abbreviations

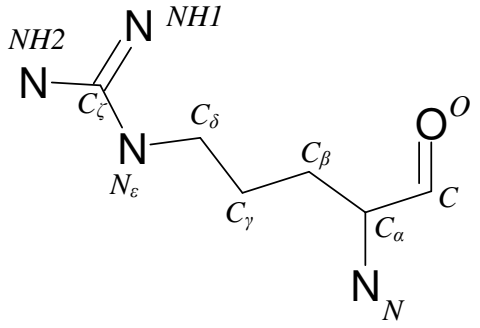
Amp	Ampicillin
BLAST	Basic Local Alignment Search Tool
ESRF	European Synchrotron Radiation Facility
FFA	Free Fatty Acids
GC	Gas Chromatography
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIC	Hydrophobic Interaction Chromatography
IEX	Ion Exchange Chromatography
IMAC	Immobilised Metal Affinity Chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Daltons
LAB	Lactic Acid Bacteria
LB	Luria-Berteli Broth / Luria Broth / Lysogeny Broth
MAD	Multiple Anomalous Dispersion
MCS	Multiple Cloning Site / polylinker
MR	Molecular Replacement
MWt	Molecular Weight
NaAc	Sodium acetate
NCBI	National Centre for Biotechnology Information
nuc	Nucleophile
OD	Optical Density
ORF	Open Reading Frame
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
SAD	Single Anomalous Dispersion
SEC	Size Exclusion Chromatography
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
Sp.	Species
SSRL	Stanford Synchrotron Radiation Laboratory
Subsp.	Subspecies
TRIS	Tris (hydroxymethyl) Aminomethane
WT	Wild Type

Amino Acid Abbreviations

Amino acid	One letter code	Three letter code
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartate	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamate	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

Atom Numbering

This thesis uses the atom numbering system used by the Protein Data Bank (PDB). Greek letters are used to denote each atom outwards along the side chain from the C_α, with numbers used to differentiate between atoms where branching occurs. Examples are given below. It should be noted that in .pdb files, Roman letters are substituted for their Greek equivalents.



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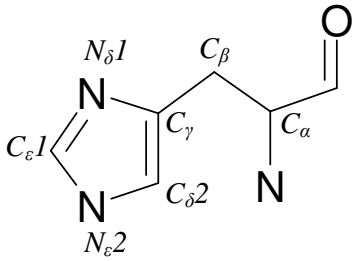

Histidine

Nucleic Acid Abbreviations

One Letter Code	Base(s) Represented
A	Adenosine
C	Cytosine
G	Guanine
T	Thymidine
U	Uridine / Uracil
R	G or A
Y	T or C
K	G or T
M	A or C
S	G or C
W	A or T
B	G or T or C
D	G or A or T
H	A or C or T
V	G or C or A
N	Any