Structural and functional studies of pseudomurein peptide ligases in methanogenic archaea

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Abstract

Prokaryotes are classified as *Archaea* and *Bacteria* in the tree of life and have several distinguishing characteristics, among which the cell wall is one of the most essential and early evolving. Cell walls serve a number of essential functions including protection against osmotic stress, maintenance of cell shape, reduction of lateral gene transfer, and protection from viruses. The cell walls in *Bacteria* are predominantly comprised of peptidoglycan (murein) whereas *Archaea* contain a wide range of cell wall types, none of them being murein. However, methanogens of the order *Methanobacteriales* and *Methanopyrales* contain pseudomurein that shares an overall architectural structure similar to that of murein with a glycan backbone that is cross-linked by a peptide. Understanding the enzymatic steps for pseudomurein pentapeptide biosynthesis and structural information of these enzymes, could be key to resolving the evolutionary history of cell wall synthesis and was the focus of this project.

Analysis of the sequences and gene clusters of the murein peptide ligase genes suggested that analogous putative pseudomurein peptide ligases exist in methanogens. Moreover, the structures of two pseudomurein peptide ligases, pMurE and pMurC, the first of any archaeal peptide ligase, have been determined and their structural homology with bacterial murein ligase MurE and MurC, respectively, was analysed. The structures of pMurE from *Methanothermus fervidus* DSM 2088 (Mfer762) and *Methanothermobacter thermautotrophicus* ΔH DSM 1053 (Mth734), and pMurC (Mfer336), also from *M. fervidus* were determined to a resolutions of 1.7, 2.7, and 2.5 Å, respectively. The pseudomurein peptide ligase structures share a similar overall three domain arrangement and one shows a rigid-body rotation of the C-terminal domain as observed for murein peptide ligases. The ATP-binding sites in both pMurE and pMurC have been identified based on structure

homology. The N^{α}-UDP-Glu$^{\gamma}$-Ala-binding site for pMurE peptide ligase has been proposed based on the UDP-binding position suggesting a similar peptide ligation mechanism as that of MurE peptide ligase. The study thereby suggests a proposed functional role of the pseudomurein peptide ligases and proposes an evolutionary pathway for both murein and pseudomurein peptide ligases from common ancestral genes.

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Dedication

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गुरुर्ब्रह्मा गुरुर्विष्णु र्गुरुर्देवो महेश्वरः

गुरु साक्षात परब्रह्मा तस्मै श्रीगुरवे नमः

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Abbreviations

°C	Temperature in centigrade
Å	Ångström (10^{-10} m)
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPD	2-Amino-2-methyl-1, 3-propanediol
AMP-PNP	Adenylyl-imidodiphosphate
ANP	Phosphoaminophosphonic acid-adenylate ester
ATP	Adenosine tri-phosphate
Bis-tris	Bis (2-hydroxyethyl) aminotris (hydroxymethyl) methane.
BLAST	Basic Local Alignment Search Tool
BME	β-mercaptoethanol
BSA	Bovine Serum Albumin
GalNAc	*N*-acetylgalactosamine
GlcA	Glucuronic acid residue
GlcNAc	*N*-acetylglucosamine
GlyGly	Glycylglycine
IPTG	Isopropyl β-D-1-thiogalactopyranoside
K	Temperature in Kelvin
KCl	Potassium chloride
kDa	Kilodalton
MOPS	3-(N-Morpholino) propane sulfonic acid
MCWO	Molecular weight cut off
mM	Millimolar (10^{-3} Molar concentration)

Mur	Murein ligase
MurNAc	*N*-acetylmuramic acid residues
NAcAltNA	*N*-acetylaltrosaminuronic acid
NAcTalNA	*N*-acetyl-L-talosaminuronic acid
NDSB	Non detergent sulfobetaines
PAGE	Polyacrylamide gel electrophoresis
pH	Measurement of acidity or basicity of an aqueous solution
P_i	Inorganic phosphate
PSI-BLAST	Position-specific iterated BLAST
PMSF	Phenylmethylsulfonyl fluoride
pMur	Pseudomurein peptide ligase
RMSD	Root-mean-square deviation
POP	Pyrophosphate representation in the protein structure
SDS	Sodium dodecyl sulfate
TCEP	Tris (2-carboxyethyl) phosphine hydrochloride
TEA	Triethanolamine
UAG	UDP-*N*-acetylmuramoyl-L-Ala-D-Glu
UDP	Uridine 5′-diphosphate
UMA	UDP-*N*-acetylmuramoyl-L-Ala
v/v	Volume per volume