Review Article

Two Major Archaeal Pseudomurein Endoisopeptidases: PeiW and PeiP

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PeiW (UniProtKB Q7LYX0) and PeiP (UniProtKB Q77WJ4) are the two major pseudomurein endoisopeptidases (Pei) that are known to cleave pseudomurein cell-wall sacculi of the members of the methanogenic orders Methanobacteriales and Methanopyrales. Both enzymes, originating from prophages specific for some methanogenic archaeal species, hydrolyze the ϵ(Ala)-Lys bond of the peptide linker between adjacent pseudomurein layers. Because lysozyme is not able to cleave the pseudomurein cell wall, the enzymes are used in protoplast preparation and in DNA isolation from pseudomurein cell-wall-containing methanogens. Moreover, PeiW increases the probe permeability ratio and enables fluorescence in situ hybridization (FISH) and catalyzed reporter deposition (CARD-) FISH experiments to be performed on these methanogens.

1. Introduction

Bacterial peptidoglycan hydrolases are among the most extensively studied hydrolases. Many three-dimensional structures of murein hydrolases have already been deposited in the Protein Data Bank; on the other hand, pseudomurein hydrolases are very poorly studied. Pseudomurein, the major cell wall component of gram-positive methanogenic archaea, is composed of N-acetyl-D-glucosamine (NAG) and N-acetyl-L-talosaminuronic acid (NAT) linked by a β(1 → 3)-glycosidic bond (see Figure 1) [1, 2]. Therefore, it is resistant to lysozyme and other bacterial hydrolases. The other cell wall polymers of methanogens include methanochondroitin and (glyco)protein surface (S)-layers [3]. Not much is known with regard to cell-wall-degrading lysins in methanogenic archaea. Pseudomurein endoisopeptidase (Pei) is the first enzyme known to hydrolyze the pseudomurein sacculi of archaeal methanogens [2, 4–7].

This short review not only gathers the available data on this unique enzyme but also enlightens its future research prospective. There is no three-dimensional structure of this enzyme despite its novelty and application potential. Further, research leading to the elucidation of the three-dimensional structure of pseudomurein endoisopeptidase may substantially increase our knowledge of its functionalities and possible applications to commercially and environmentally important methanogens.

2. The Pei Enzyme

Pseudomurein endoisopeptidase belongs to peptidase family C71, which contains peptidases that cleave the peptide subunits of pseudomurein sacculi (MEROPS database, http://merops.sanger.ac.uk/cgi-bin/famsum?family=C71). Pei is absent in most model organisms; so far, it has only been found in two prophages specific for some methanogenic archaeal species, Methanobacteriales and Methanopyrales. Both enzymes, originating from prophages specific for some methanogenic archaeal species, hydrolyze the ϵ(Ala)-Lys bond of the peptide linker between adjacent pseudomurein layers. Because lysozyme is not able to cleave the pseudomurein cell wall, the enzymes are used in protoplast preparation and in DNA isolation from pseudomurein cell-wall-containing methanogens. Moreover, PeiW increases the probe permeability ratio and enables fluorescence in situ hybridization (FISH) and catalyzed reporter deposition (CARD-) FISH experiments to be performed on these methanogens.
and modified from Kiener et al. [4].

cleavage site of Pei in the peptide subunit. The picture was adapted from the pseudomurein endoisopeptidase (Pei). Block arrows indicate the pseudomurein binding repeat domain (PMBR) (pfam 09373)

The domain organization of PeiP and PeiW indicates that they contain two distinct domains, an N-terminal pseudomurein binding repeat domain (PMBR) and a C-terminal catalytic cysteine protease domain (pfam 12386) (see Figure 2) [7, 11].

3.1. The N-Terminal Pseudomurein Binding Repeat Domain. The PMBR domains are not only present in PeiW and PeiP but also in 24 archaeal and 7 bacterial proteins (Interpro, http://www.ebi.ac.uk/interpro/Entry?ac=IPR018975). The PMBR domains of PeiW and PeiP contain four direct repeats, each of which contains 30 to 35 amino acid residues (see Figure 2) [http://www.ebi.ac.uk/interpro/ISpy?ipr=IPR022119&tax=35237] [8]. As deletion of the PMBR domain in PeiW resulted in loss of binding of the enzyme to the pseudomurein layer, the domain is apparently involved in the binding of the enzyme to pseudomurein [11]. By deleting 1, 2, or 3 repeats from the PMBR domain of the surface (S)-layer protein MTH719 of Methanothermobacter thermautotrophicus, we could show that binding of the domain to pseudomurein only takes place if a minimum of three repeats are present [13]. The repeat structure of the binding domain is consistent with the polymeric nature of the substrate and probably serves as a determinant of substrate specificity [7]. The molecular function of the PMBR domain can thus be compared to that of the major murein binding domain, LysM, which is commonly found in cell wall hydrolases of bacteria and in other proteins [14, 15]. Steenbakkers et al. [11] have shown that PeiW hydrolyzes the pseudomurein layer by being associated with it, a process that is aided by the PMBR domain.

3.2. The C-Terminal Catalytic Domain. The catalytic endoisopeptidase domain of both PeiW and PeiP is involved in the cleavage of the linker peptide connecting the adjacent pseudomurein layers [6, 10]. When this domain was removed from PeiW or PeiP, the truncated enzymes did not lyse Methanothermobacter sp. cells [11]. The catalytic domain possesses a catalytic triad consisting of a cysteinyl residue, a histidyl residue, and an aspartyl residue (C-H-D) (see Figure 2) [7]. As the catalytic triad has a cysteinyl residue as putative nucleophile, PeiW and PeiP are highly sensitive to oxidative agents [7]. As discussed by Pfister and coworkers [10], PeiP catalyzes the in vitro lysis of Methanothermobacter thermautotrophicus Marburg cells only under anaerobic and reducing conditions [7, 10]. The catalytic triad C-H-D is homologous to that in animal transglutaminases like human blood clotting factor XIII and thiol proteases such as papain [7, 16].

4. Homologies

BLAST [17] searches revealed that PeiW and PeiP are very closely related to each other. An amino acid sequence comparison showed that the two enzymes have 53.4% identical amino acid residues and that the regions surrounding the catalytic triad residues are especially well conserved (see Figure 2) [6]. As mentioned earlier, the PeiW-related protein (UniProtKB A5UNW8) and a putative uncharacterized protein (UniProtKB D2ZMY6), from two different strains of Methanothermobacter smithii, do not have an N-terminal PMBR domain, but...
they do carry a C-terminal domain in which the amino acids surrounding the catalytic triad C-H-D are well conserved [8].

5. Applications of Pei

Lack of permeability of whole cells for oligonucleotide probes is always a major hurdle for the in situ detection of methanogens of the orders Methanobacteriales and Methanopyrales [2]. The probe hybridization ratio can be dramatically increased by treatment of cells with Pei, allowing for a very efficient analysis of methanogens by fluorescence in situ hybridization (FISH) experiments [2]. Pei treatment of whole cells is employed as a novel permeabilization method for catalyzed reporter deposition-(CARD-) FISH experiments on methanogens [9]. Pei is also widely used in the large-scale purification of undegraded genomic and plasmid DNA from M. thermautotrophicus and for the preparation of protoplasts from methanogens containing a pseudomurein cell envelope [4].

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