European Journal of Medicinal Chemistry 139 (2017) 482–491

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Crystal structure of *Porphyromonas gingivalis* dipeptidyl peptidase 4 and structure-activity relationships based on inhibitor profiling

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ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form
7 August 2017
Accepted 8 August 2017
Available online 10 August 2017

Keywords:
Dipeptidyl peptidase 4
Peptidase inhibitor
Porphyromonas gingivalis
Biofilm
Dipeptidyl peptidase 9

ABSTRACT

The Gram-negative anaerobe *Porphyromonas gingivalis* is associated with chronic periodontitis. Clinical isolates of *P. gingivalis* strains with high dipeptidyl peptidase 4 (DPP4) expression also had a high capacity for biofilm formation and were more infective. The X-ray crystal structure of *P. gingivalis* DPP4 was solved at 2.2 Å resolution. Despite a sequence identity of 32%, the overall structure of the dimer was conserved between *P. gingivalis* DPP4 and mammalian orthologues. The structures of the substrate binding sites were also conserved, except for the region called S2-extensive, which is exploited by specific human DPP4 inhibitors currently used as antidiabetic drugs. Screening of a collection of 450 compounds as inhibitors revealed a structure-activity relationship that mimics in part that of mammalian DPP9. The functional similarity between human and bacterial DPP4 was confirmed using 124 potential peptide substrates.

1. Introduction

Periodontal disease develops when bacteria of the dental plaque migrate from the dental root hard tissues to nearby soft tissues. This results in chronic inflammation leading to destruction of the periodontal ligament and the alveolar bone that supports the teeth. The process by which a generally benign biofilm transforms into pathological periodontitis is complex and remains poorly understood. However, the presence of one particular species, the Gram-negative anaerobe *Porphyromonas gingivalis*, is considered to be an indicator of progression of chronic periodontitis [1,2]. Periodontal disease, and specifically the presence of *P. gingivalis*, are risk factors for cardiovascular disease, aspiration pneumonia and low birth weight [3–5]. A wide array of virulence factors have been described for *P. gingivalis* that reflect the different processes by which the bacterium transforms from a commensal organism to a pathogen: biofilm formation, anaerobic growth, adhesion, suppression/activation of inflammatory responses, invasion, tissue degradation and intracellular survival in gingival epithelial cells and host immune cells (reviewed by Refs. [6,7]). *P. gingivalis* produces a unique set of proteases that are believed to be required for nutrient provision during its compulsory asaccharolytic growth, but may also contribute to evasion of the host immune system, adhesion and degradation of extracellular matrix [8–10]. One of these proteases is *P. gingivalis* dipeptidyl peptidase 4 (pgDPP4). The gene encoding pgDPP4 was first cloned and sequenced in 2000 [11], and the protein is secreted but remains associated with the membrane [12]. PgDPP4 is a proline-specific dipeptidyl peptidase that cleaves peptides related to immune responses and inflammation

Abbreviations: DPP, dipeptidyl peptidase; hDPP4, human dipeptidyl peptidase 4; MAD, Multiwavelength Anomalous Dispersion; pgDPP4, *Porphyromonas gingivalis* dipeptidyl peptidase 4; SAR, structure-activity relationship.

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http://dx.doi.org/10.1016/j.ejmech.2017.08.024
0223-5234/

in vitro. Evidence suggests pgDPP4 interacts with extracellular matrix proteins such as fibronectin and collagen, thereby promoting collagen degradation by matrix metalloproteinases activated by the host inflammatory response [13,14]. Mice injected with a DPP4-deficient mutant strain developed fewer abscesses and survived longer than mice injected with the wild type (W83) strain [11], implicating pgDPP4 as a virulence factor. Transfection with recombinant pgDPP4 restored virulence, while a catalytically impaired DPP4 only partially restored the wild type phenotype [15]. Moreover, clinical isolates of *P. gingivalis* strains with high DPP4 expression had a higher capacity for biofilm formation and were more infective in a mouse abscess model [16].

pgDPP4 belongs to the same family of proteases (prolyl oligopeptidase family, S9 clanB, MEROPS database) as human DPP4 (hDPP4), with which it shares 32% amino acid sequence identity. This suggests a similar topology and enzymatic mechanism. Even though the catalytic residues and side chains forming the primary substrate-binding site are conserved, we noticed significant differences in the potency of selected inhibitors, and in the substrate specificity, between the bacterial and human enzymes [17]. Following preliminary crystallisation experiments [18], one of the aims of the present study was to identify the origins of these differences in the structure of pgDPP4, as a prerequisite to developing more specific inhibitors in the future. Both enzymes likely share substrates present in the oral cavity, the gingival epithelium and at sites of inflammation.

In humans, the inhibition of DPP4-catalyzed inactivation of incretins (intestinal peptides regulating insulin secretion) proved to be successful for the treatment of type 2 diabetes [19]. Over the last two decades, numerous DPP4 inhibitors have been synthesized, screened and tested for their antidiabetic activity, while other compounds have been prepared to address selectivity and to investigate the roles of several related peptidases. Thus, there exists a sizable collection of mammalian DPP family inhibitors available for screening against other targets. The collection used in the present work includes compounds that have been developed and characterized at the University of Antwerp during a long-standing research program on various peptidases, including DPP4, DPP2, DPP8, DPP9, fibroblast activating protein-α (FAP) and prolyl oligopeptidase (PREP). They are based on several chemical scaffolds [20–33]. The collection also includes reference compounds, such as the antidiabetic drugs sitagliptin, vildagliptin and linagliptin, and prototypical DPP2, DPP8/9 and FAP inhibitors. The aim of this study was to screen the collection of compounds present at the University of Antwerp. Additionally, an assortment of secreted peptides present at the University of Leuven was used as a substrate collection in order to investigate structure-function relationships in the active site of pgDPP4.

2. Results

2.1. Structural alignment of pgDPP4 and hDPP4

The overall structural similarity between both DPP4s is shown in Fig. 1. A sequence alignment with species-specific numbering is shown in the supplementary material.

Fig. 2 depicts the catalytic residues of pgDPP4 positioned in the electron density map. The active sites of pgDPP4 and hDPP4 are evidently similar. Direct transfer of the coordinates of the hDPP4 structures with different ligands into the active site of the aligned pgDPP4 structure (*i.e.* docking of ligands in pgDPP4 active site) reveals similar substrate binding pockets for both DPP4 enzymes. Table S1 in the supplementary material provides a list of all hDPP4 structures used in this work, together with references to the Protein Structure Database (PDB) and the original publications.

A narrow but deep S1-pocket is located next to the catalytic Ser-593 (adopting pgDPP4 numbering), and direct mapping of the many co-crystallized ligands from the human structures into the pgDPP4 structure reveals that this pocket could potentially accommodate a proline-moiety like the pyrrolidine ring of the Val-

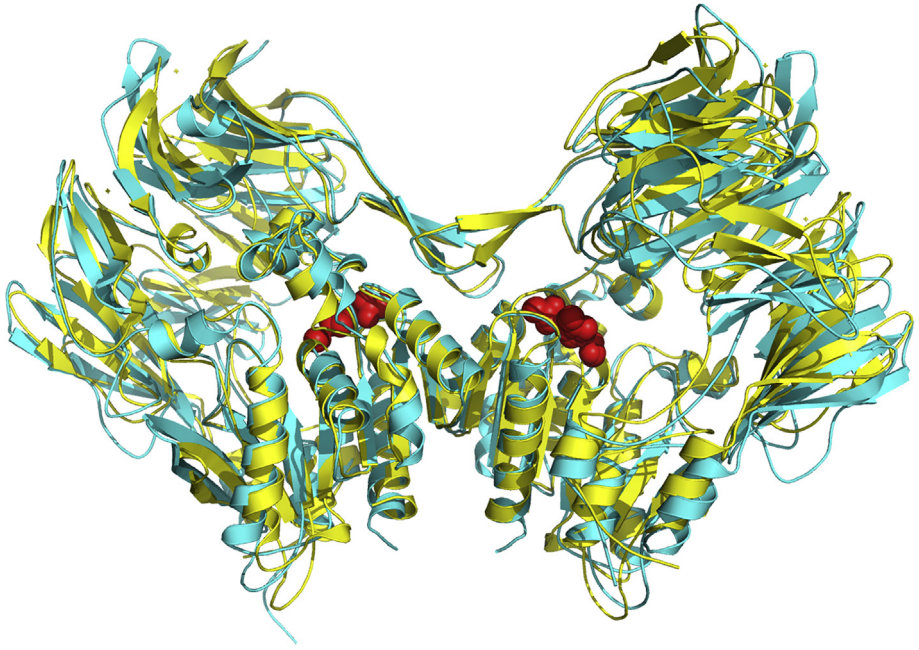

Fig. 1. Overall structural alignment of pgDPP4 and hDPP4.
Superposition of the crystal structures of human (cyan) and *P. gingivalis* (yellow) DPP4. The structure of the human form was taken from Grimshaw and coworkers (PDB ID 5KBY) [63]. Both structures are composed of two homodimers that are related by a two-fold axis. The catalytic triad residues in both structures are indicated with solid red spheres. Root-mean-squared deviation between the Cα atoms of both structures is 1.0 Å, indicating an extremely high level of structural similarity between the two proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)