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Role of a propeller loop in the quaternary structure and enzymatic activity of prolyl dipeptidases DPP-IV and DPP9

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ABSTRACT

The dipeptidyl peptidase (DPP) family members, including DPP-IV, DPP8, DPP9 and others, cleave the peptide bond after the penultimate proline residue and are drug target rich. The dimerization of DPP-IV is required for its activity. A propeller loop located at the dimer interface is highly conserved within the family. Here we carried out site-directed mutagenesis on the loop of DPPIV and identified several residues important for dimer formation and enzymatic activity. Interestingly, the corresponding residues on DPP9 have a different impact whereby the mutations decrease activity without changing dimerization. Thus the propeller loop seems to play a varying role in different DPPs.

Structured summary of protein interactions:
DPP-IV and **DPP-IV** physically interact by comigration in gel electrophoresis (View interaction: 1, 2, 3, 4)
DPP9 and **DPP9** bind by circular dichroism (View interaction)
DPP-IV and **DPP-IV** bind by circular dichroism (View interaction: 1, 2, 3, 4, 5)
DPP-IV and **DPP-IV** bind by cosedimentation in solution (View interaction: 1, 2, 3, 4, 5)
ADA binds to **DPP-IV** by surface plasmon resonance (View interaction: 1, 2, 3, 4, 5, 6)
DPP9 and **DPP9** bind by cosedimentation in solution (View interaction)

1. Introduction

The prolyl-cleaving dipeptidase family are serine proteases including dipeptidyl peptidase IV (DPP-IV), fibroblast activation protein (FAP), DPP8, and DPP9 [1]. They preferentially cleave the peptide bond after the penultimate proline residue. This unique activity distinguishes this family from most cellular enzymes, and makes them important to various biological functions in vivo [1]. The most well-studied member of the family is DPP-IV (EC 3.4.14.5), which is a validated drug target for human type II diabetes [2]. It is involved in the degradation of two insulin-sensing hormones, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide [3,4]. Chemical inhibitors of DPP-IV are effective at prolonging the half-life of these hormones and can be used in the treatment of diabetes [5]. In addition, DPP-IV also plays an important role in the regulation of adenosine signaling and in potentiating T-cell proliferation through interacting with adenosine deaminase (ADA) [6]. Determining the structure and mechanism of DPP-IV will help elucidate not only its function, but those of other prolyl cleaving enzymes, whose functions are largely unknown.

DPP-IV is a membrane protein with a short cytoplasmic tail and a transmembrane domain (residues 1–28). The crystal structure of the ectodomain (residues 29–766) has revealed that it is homodimeric [7] (Fig. 1A). It contains an α/β-hydrolase domain and a β-propeller domain, with the active site located between them. The dimeric interface included the C-terminal loop from the hydrolase domain and the β-propeller loop from the propeller domain. Previously, we have shown that a single mutation within the C-terminal loop disrupts DPP-IV dimerization with a concomitant loss of enzymatic

Abbreviations: DPP-IV, dipeptidyl peptidase IV; DPP, dipeptidyl peptidase; POP, prolyl oligopeptidase; H-Gly-Pro-pNA, HGly-Pro-p-nitroanilide; DTT, dithiothreitol; AUC, analytical ultracentrifugation; CD, circular dichroism; FAP, fibroblast activation protein; ADA, adenosine deaminase; SPR, surface plasmon resonance.

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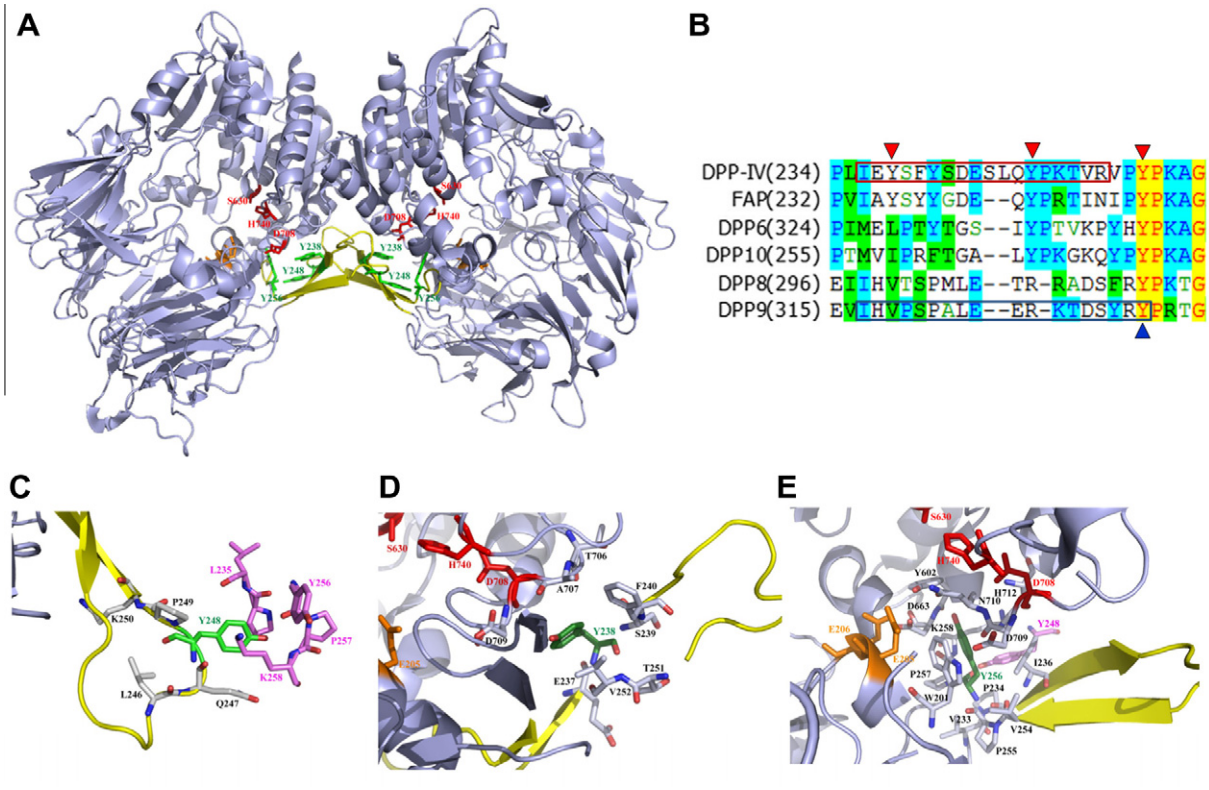

Fig. 1. Schematic representation of the residues in the propeller loop of DPP-IV. (A) DPP-IV structure (1N1 M) with the propeller loop highlighted in yellow. Residues Y248, Y238 and Y256 are shown as green sticks. The catalytic triad S630-H740-D708 is represented in red and the D-D motif as orange. (B) Sequence alignment of the propeller loops from DPPs. GenBank accession numbers: NP_001926 (DPP-IV), Q12884 (FAP), NP_570629.2 (DPP6), AAG29766 (DPP8), NP_631898.3 (DPP9) and P42658 (DPP10) (C–E) Detailed interactions of Y248, Y238 and Y256 of DPP-IV with neighboring residues. The residues on the same subunit are shown in silver, and those on the opposite subunit in pink. The figures were drawn with PyMOL [28].

activity [8,9]. We also have observed that no equilibrium existing between the dimer and monomer states of DPP-IV, indicating that the monomer conformation is different enough to prevent direct association with another monomer [8,9]. Until now, the contributions of the propeller loop to DPP-IV are unknown.

Several other members of the DPP family have been identified with a various cellular functions. DPP8 and DPP9 are also dimeric with DPP activity [10–13]. Unlike DPP-IV, they are found in the cytosol. DPP9 is the major DPP in the cytoplasm and responsible for at least 80% of the cleavage activity measured using dipeptide substrates [12]. Recently it has been found to be important to immune function [12]. Another member, FAP, is highly homologous with DPP-IV. It has been implicated as a potential anti-cancer target [14]. Studying the structure and activity of these enzymes is important to our understanding of their function in vivo.

The propeller loop is highly conserved among DPP-IVs from different species (Supplemental Fig. 1A) and moderately conserved among the DPP family as a whole (Fig. 1B). It is an anti-parallel two-stranded β-sheet, extending from blade 4 in the propeller domain and interacting with the same loop of another subunit (Fig. 1A) [7]. DPP-IV has the longest propeller loop (residues 234–260), while the loops from others are two to three residues shorter. Intriguingly, based on the structures, the propeller loops from the DPP family are generally longer than those from other prolyl-cleaving endopeptidases such as the prolyl oligopeptidases (POPs) [15], which are 12 to 15 amino acids long (Supplemental Fig. 1B). In this context, DPP-IV is only active as a dimer, while the POPs are active as a monomer. Whether the length of the propeller loop plays a part in the dimerization of DPP family remains unclear.

Despite speculation [7], the function of the DPP-IV propeller loop in terms of quaternary structure and enzymatic activity has not been studied as yet. Here we investigated the contribution of the propeller loop of DPP-IV to these areas using site-directed mutagenesis. Moreover, to understand whether the propeller loop also affects other DPPs, we assessed the effect of similar mutations targeting the putative propeller loop of DPP9.

2. Materials and methods

2.1. Materials

The human liver cDNA library and viral vector were obtained from Clontech (Mountain View, CA, USA) and fetal bovine serum, lipofectamine, and insect culture media from Invitrogen (Carlsbad, CA, USA). The Western detection kit was obtained from Perkin-Elmer (Waltham, Massachusetts, USA) and bovine ADA from Roche (Branchburg, NJ, USA). Size-exclusion column, CM5 chip, and the nickel affinity column were produced by GE healthcare (Uppsala, Sweden). Amicon filter with 10 kDa-cut was bought from Millipore (Billerica, MA, USA). Ala-Pro-pNA was purchased from Bachem (Torrance, CA, USA).

2.2. Plasmid construction, insect and mammalian cell culture

To construct the plasmids with mutations for expression in insect cells, the baculovirus expression plasmid pBac-CD5-DPPIV (residues 39–766) [8] and pBacMTeGFP-StrepDPP9 (StrepTagII in N-terminus) [11] were used. Site-directed mutagenesis was performed using quick-change mutagenesis [8,9]. The primers used are listed in Supplemental Table 1. The nucleotide sequences were confirmed by autosequencing analysis. Sf9 and Hi5 cells were grown in TNM-FH medium supplemented with 10% fetal bovine serum and EXPRESS FIVE® SFM medium, respectively, at 27 °C