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Crystallization and preliminary X-ray study of human dipeptidyl peptidase IV (DPPIV)

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Human DPPIV has been expressed in the baculovirus system and purified and crystallized using the hanging-drop method. A crystal was obtained from 180 m*M* Gly–NaOH buffer pH 9.5 containing 18% PEG 4000 and 180 m*M* sodium acetate. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 118.04, b = 125.92, c = 136.84 Å, and diffracts beyond 2.6 Å resolution. There are two molecules per asymmetric unit, indicating a solvent content of 57.6%.

1. Introduction

Serine proteases are abundant in living cells and play important roles in intracellular proteolysis (Neurath, 1984). The POP (prolyl oligopeptidase) family, a member of the serine proteases, consists of prolyl endopeptidase (EC 3.4.21.26; Cunningham & O'Connor, 1997), oligopeptidase B (EC 3.4.21.83; Pacaud & Richaud, 1975), DPPIV (EC 3.4.14.5; Frohman *et al.*, 1989; Misumi *et al.*, 1992; Duke-Cohan *et al.*, 1995; Dobers *et al.*, 2002) and acylpeptide hydrolase (EC 3.4.19.1; Mitta *et al.*, 1989). DPPIV and POP have amino-acid identities of 21% in the peptidase domain and ≤15% in other regions (Abbott *et al.*, 1999). DPPIV is widely expressed in a number of mammalian tissues and is a cell-surface type II membrane glycoprotein; it is also referred to as adenosine deaminase (ADA) binding protein or CD26, which is known as a T-cell activation antigen (Bednarczyk *et al.*, 1991). DPPIV is implicated in several diseases such as inflammation and diabetes (Valenzuela *et al.*, 1997; Vilsboll *et al.*, 2001). Since the overactivation of DPPIV leads to serious disease, development of selective inhibitors that may be useful as drugs or as reagents for further characterization of DPPIV is of interest.

Previously, the crystal structures of POP from porcine muscle and brain have been determined at high resolution (Fülöp *et al.*, 1998, 2001). The structure consists of an N-terminal domain, which possesses an unusual seven-bladed β-propeller structure, and a C-terminal catalytic protease domain. With regard to the propeller structure, four- to eight-propeller folds have been reported in several proteins (Fülöp & Jones, 1999; Jawad & Paoli, 2002). In the POP family, only the seven-bladed β-propeller structure has been reported.

To clarify the relationship between the biological function and the domain structural features of human DPPIV, we have initiated structure determination by X-ray crystallography. Here, we report the crystallization and preliminary crystallographic data.

2. Methods and results

2.1. Protein production in insect cells

The cDNA fragment encoding the soluble form (region 33–766) of human DPPIV (SWISS-PROT accession No. P27487) and including the insertion of a hexahistidine tag at the C-terminal was amplified by RT-nested PCR using total RNA isolated from Caco-2 cells as a template. The PCR-generated 2.2 kbp DNA fragment was separated on AGE and subcloned into a TOPO TA-cloning vector, pCR 2.1-TOPO (Invitrogen), designated pCR-shDPPIV. The baculovirus transfer vector, pAcGP67B-shDPPIV, was constructed by subcloning a *Bam*HI–*Eco*RI fragment of pCR-shDPPIV into the corresponding sites of pAcGP67B (PharMingen). Recombinant baculovirus was prepared according to the instruction manual. Sf21 cells (Invitrogen) were grown in an EX-CELL 400 serum-free medium (JRH Biosciences) at 301 K. The insect cells were infected with recombinant baculovirus stocks at a multiplicity of infection of about two. 4 d after infection, the supernatant was collected and stored at 253 K until use.

2.2. Purification

The supernatant was filtrated with a 0.45 µm filter (Kurabo) to remove insoluble materials. The filtrate was concentrated and dialyzed against buffer *A* (20 m*M* HEPES–NaOH, 0.5 *M* NaCl pH 8.0) overnight at 277 K. Dialyzed sample was applied to a HiTrap Chelating column (5 ml, Pharmacia) previously immobilized with nickel and equilibrated with

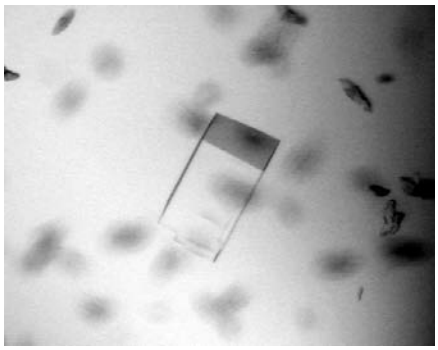

Figure 1
A photograph of a human DPPIV crystal of dimensions 0.5 × 0.3 × 0.1 mm.

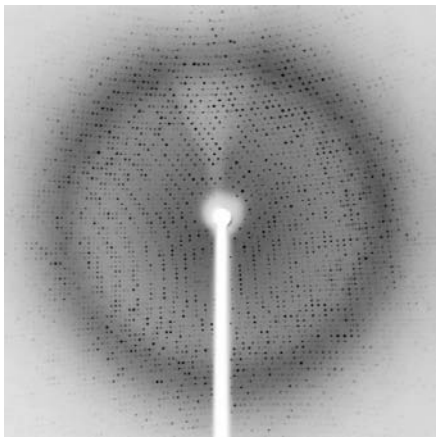

Figure 2
Diffraction pattern of a human DPPIV crystal.

buffer *A*. The column was washed with 10 column volumes of buffer *A* and then with buffer *A* containing 50 m*M* imidazole. The column was developed with 20 column volumes of a linear gradient of imidazole (50–500 m*M*). Fractions containing DPPIV activity were pooled and dialyzed against buffer *B* (20 m*M* HEPES–NaOH pH 8.0, 50 m*M* NaCl). The dialyzed fraction was rechromatographed on an anion-exchange column (Resource Q, 1 ml, Pharmacia) equilibrated with buffer *B*. The column was washed and then developed with 15 column volumes of a linear gradient of NaCl (50–500 m*M*). The active fractions were pooled. The purified DPPIV was verified in 71 m*M* Gly–NaOH buffer pH 8.7 by its proteolytic activity against a peptide substrate. The solution was concentrated to 20 mg ml^{-1} for crystallization using a Centricon 10 (Amicon). The purity of the crystallization sample was confirmed by SDS–PAGE.

Table 1
Data-collection statistics of human DPPIV.

Values given in parentheses are for the highest resolution shell (2.74–2.60 Å).

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 118.04, b = 125.92, c = 136.84
V_M† (Å^3 Da^{-1})	2.88
V_s‡ (%)	57.6
Z	8
Resolution (Å)	2.6
No. of observations	385015
No. of unique reflections	58657
Completeness (%)	95.0 (72.8)
R_{merge}§ (%)	7.8 (21.3)
Redundancy	6.4 (4.6)
$I/\sigma(I)$	7.5 (3.5)

† $V_M = V_c/ZM$, where V_c is the unit-cell volume and M is the molecular weight. ‡ Solvent content $V_s = 1 - 1.23/V_M$. § $R_{merge} = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I(hkl)_i$.

2.3. Crystallization

Crystals of human recombinant DPPIV were grown by vapour diffusion at 293 K using the sitting-drop method. Droplets of 10 µl initial volume were prepared on siliconized glass cover slips suspended over reservoirs. Crystallization was attempted using Hampton Crystal Screens I and II and Wizard crystal screening kits I and II (Emerald BioStructures, Inc.). Crystals were finally obtained from 180 m*M* Gly–NaOH buffer pH 9.5 containing 180 m*M* sodium acetate and 18% PEG 4000. Plate-shaped crystals with maximum dimensions of 0.5 × 0.3 × 0.1 mm appeared after two weeks using a seeding method (Fig. 1).

2.4. Data collection

A single crystal sealed with 15% glycerol as a cryoprotectant was mounted in a Cryoloop (Hampton Research) under an N_2 stream at 100 K and its X-ray diffraction data were collected on an R-AXIS IV imaging-plate detector with a Rigaku rotating-anode X-ray source using Cu $K\alpha$ radiation equipped with an Osmic mirror system. The crystals were relatively well ordered, with little sign of decay during the X-ray exposure. All data were processed and scaled using the program *MOSFLM* v.6.0 (Leslie, 1992).

Crystal data are reported in Table 1. The calculated V_M, assuming two molecules in the asymmetric unit, is 2.88 Å^3 Da^{-1} (Matthews, 1968). A native data set was collected to 2.6 Å resolution with an oscillation angle of 1.0° per frame (for a total of 180 frames; Fig. 2). A summary of the data-collection statistics is given in Table 1. The crystal structure determination is now in progress.

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