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Dipeptidyl Peptidase IV from *Xanthomonas maltophilia*: Sequencing and Expression of the Enzyme Gene and Characterization of the Expressed Enzyme[1]

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The dipeptidyl peptidase IV [EC 3.4.14.5] gene of *Xanthomonas maltophilia*, expressed in *Escherichia coli*, was cloned by the shotgun method. Nucleotide sequence analysis revealed an open reading frame of 2,223 bp, coding for a protein of 741 amino acids with a predicted molecular weight of 82,080. The expressed enzyme was extracted with SDS, and the solubilized enzyme was purified about 1,030-fold on columns of Toyopearl HW65C, DEAE-Toyopearl twice, and hydroxyapatite, with an activity recovery of 50%. The enzyme hydrolyzed a proline-containing peptide at the penultimate position, and was inhibited by diisopropyl phosphofluoridate. The enzyme was most active at pH 8.5, and was stable between at pH 7.0 and 9.0. The molecular weight of the purified enzyme was estimated to be 83,000 and 165,000 by SDS-PAGE and gel filtration, respectively.

Key words: α/β hydrolase fold family, cloning, crystallization, dipeptidyl peptidase IV, serine protease.

Dipeptidyl peptidase IV (DP IV; EC 3.4.14.5) catalyzes the hydrolytic removal of N-terminal dipeptidyl residues from peptides containing proline in the penultimate position (*1*).

It is a membrane-bound enzyme, which has been isolated from various mammalian tissues (*2-4*), as well as from bacteria such as *Flavobacterium meningosepticum* (*5*), *Aspergillus oryzae* (*6*), and *Lactobacilli* (*7*). The enzyme is of interest as it cleaves X-proline dipeptides from the NH_2 terminus of several bioactive peptides, such as growth hormone-releasing hormone (*8*) and substance P (*9, 10*). In addition to its peptidase activity, DP IV has been identified as being CD26, a surface differentiation marker involved in the transduction of mitogenic signals in thymocytes and T lymphocytes in mammals (*11-13*), and in cell matrix adhesion through specific interactions with fibronectin and collagen (*14, 15*).

DP IV is classified as a member of the prolyl oligopeptidase family, which differs from classical serine protease families such as chymotrypsin and subtilisin (*16-18*). The prolyl oligopeptidase family also includes protease II (*19*) and serine carboxypeptidase II (*20*). It is well known that the active residues of these serine proteases are composed of a histidine-acid-serine catalytic triad. The sequential order of the catalytic triad residues of members of the prolyl oligopeptidase family is Ser-Asp-His, while for members of the chymotrypsin and subtilisin families, it is His-Asp-Ser and Asp-His-Ser, respectively.

Besides these serine proteases, lipases, esterases, and some hydrolases also have a histidine-acid-serine catalytic triad, the sequential order of which is Ser-Acid-His, the same as for prolyl oligopeptidase family enzymes. Although no clear primary structure similarity has been detected among lipases, esterases, serine carboxypeptidases and hydrolases, three-dimensional analyses of these enzymes indicate that they belong to an α/β hydrolase fold family (*20-24*).

To provide the basis for detailed studies on the structural relationship of DP IV with enzymes of the α/β hydrolase fold family, DP IV gene was cloned from *Xanthomonas maltophilia*. In this paper, we report on the nucleotide sequence of the DP IV gene from *X. maltophilia*, and describe the crystallization of the enzyme expressed in *E. coli*.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media—*E. coli* DH1, DH5α, and JM105 were used as hosts for cloning. Plasmids pUC18, pUC19, and pBluescript SK(+) were used as vectors. Bacteria were grown in Luria-Bertani broth (LB-broth).

Materials—Restriction endonucleases and various DNA modifying enzymes were purchased from Takara Shuzo and Toyobo. ⊿Tth DNA Polymerase Sequencing PRO was from Toyobo. [α-^{32}P]dCTP was from Amersham. Agarose I was from Dojin Chemicals, and calf intestine alkaline phosphatase was from Boehringer-Mannheim.

Preparation of Gene Library and Screening of the Enzyme Gene—*X. maltophilia* chromosomal DNA was

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Abbreviations: DP IV, dipeptidyl peptidase IV; DP B, dipeptidyl aminopeptidase B; XPDA, X-prolyl dipeptidylaminopeptidase; Gly-Pro-2NNap, Gly-Pro-β-naphthylamide; DFP, diisopropyl phosphofluoridate; PCMB, *p*-chloromercuribenzoate; PMSF, phenylmethanesulfonyl fluoride.

prepared by the method of Saito and Miura (*25*), digested with *Bam*HI, and ligated into the dephosphorylated *Bam*HI site of pUC19. The ligated plasmid mixture was used to transform *E. coli* JM105. The transformants were screened for the expression of the DP IV gene by measuring activity with Gly-Pro-2NNap as the substrate, in a 96-well polystyrene assay plate.

Subcloning and Nucleotide Sequencing—Restriction endonuclease fragments of chromosomal DNA were subcloned into pUC18, pUC19, or pBluescript SK(+), following standard procedures (*26*). Nucleotide sequencing was carried out by the dideoxy chain termination method.

Assay of Dipeptidyl Peptidase IV Activity—DP IV activity was assayed with Gly-Pro-2-NNap as the substrate, according to the method of Yoshimoto and Tsuru (*5*).

To 800 μl of 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, 100 μl of 5 mM Gly-Pro-2-NNap, and 100 μl of enzyme sample were added. After 5 min of incubation at 37°C, the enzyme reaction was stopped by adding 1 ml of Fast Garnet GBC salt (1 mg/ml) solution containing 10% Triton X-100 in 1 M acetate buffer (pH 4.0). Absorbance at 550 nm was measured after incubation for 10 min at room temperature. One unit of activity was defined as the amount of enzyme which released 1 μmol of β-naphthylamine per min under standard conditions.

Purification of the Expressed Enzyme—*E. coli* DH1/pXDP, that carried a 3.2 kbp insert of chromosomal DNA at the *Bam*HI site of pUC 19 (Fig. 1), was aerobically cultured in 12 liter N-broth containing ampicillin (50 mg/liter) at 37°C for 12 h, using a New Brunswick jar fermenter. Cells were harvested by centrifugation, washed with 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, and resuspended in 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA and 0.1% SDS. The cells were disrupted with glass beads in a Dyno-Mill, and the suspension was centrifuged at 8,000 rpm for 30 min. After centrifugation, the supernatant was saturated with 40% ammonium sulfate and applied to a column of Toyopearl HW65C (6×15 cm) equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA and ammonium sulfate at 40% saturation. The column was washed with the same buffer, and adsorbed enzymes were eluted with a decreasing linear gradient of ammonium sulfate concentration from 40 to 0% saturation

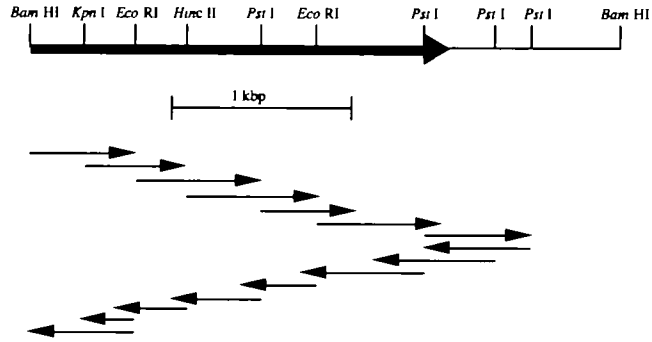

Fig. 1. **Restriction endonuclease map of the 3.2 kbp chromosomal DNA fragment.** The large arrow indicates the position and direction of the enzyme gene, and small arrows indicate the direction and extent of each sequence determination.

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                                                                  GGATCCTCC    -1
ATGCGCCATCTGTTCGCCTCGCTCGCCTTCATGCTCGCCACCAGTACCGTCGCCCACGCTGAAAAACTCACCCTGGAAGCCATCACCGGCCCGCTGCCGCTGTCCGGCCCGACCCTGATG   120
M  R  H  L  F  A  S  L  A  F  M  L  A  T  S  T  V  A  H  A  E  K  L  T  L  E  A  I  T  G  P  L  P  L  S  G  P  T  L  M
AAGCCGAAGGTGGCGCCGGACGGCTCGCGGGTGACCTTCCTGCGCGGCAAGGACAGCGACCGCAACCAGCTGGACCTGTGGAGCTACGACATCGGCAGCGGTCAGACCCGCTTGCTGGTG   240
K  P  K  V  A  P  D  G  S  R  V  T  F  L  R  G  K  D  S  D  R  N  Q  L  D  L  W  S  Y  D  I  G  S  G  Q  T  R  L  L  V
GACTCGAAGGTGGTGCTGCCGGGTACCGAAACCCTCAGTGACGAAGAAAAGGCCCGCCGCGAGCGCCAGCGCATCGCGGCGATGACCGGCATCGTCGATTACCAGTGGTCGCCGGACGCG   360
D  S  K  V  V  L  P  G  T  E  T  L  S  D  E  E  K  A  R  R  E  R  Q  R  I  A  A  M  T  G  I  V  D  Y  Q  W  S  P  D  A
CAGCGCCTGCTGTTCCCGCTGGGCGGCGAGCTGTACCTGTACGACCTCAAGCAGGAAGGCAAGGCGGCAGTGCGCCAGCTGACCCACGGCGAAGGCTTTGCGACCGATGCCAAGCTGTCG   480
Q  R  L  L  F  P  L  G  G  E  L  Y  L  Y  D  L  K  Q  E  G  K  A  A  V  R  Q  L  T  H  G  E  G  F  A  T  D  A  K  L  S
CCCAAGGGCGGCTTCGTCAGCTTCATCCGCGGCCGCAACCTGTGGGTGATCGACCTGGCCAGCGGCAGGCAGATGCAGCTGACCGCTGATGGCAGCACGACCATCGGCAACGGTATCGCC   600
P  K  G  G  F  V  S  F  I  R  G  R  N  L  W  V  I  D  L  A  S  G  R  Q  M  Q  L  T  A  D  G  S  T  T  I  G  N  G  I  A
GAATTCGTCGCCGACGAAGAAATGGACCGCCATACTGGTTACTGGTGGGCGCCGGACGATTCGGCCATCGCATACGCCCGCATCGATGAAAGCCCGGTGCCGGTGCAGAAGCGTTACGAA   720
E  F  V  A  D  E  E  M  D  R  H  T  G  Y  W  W  A  P  D  D  S  A  I  A  Y  A  R  I  D  E  S  P  V  P  V  Q  K  R  Y  E
GTCTATGCCGACCGCACCGATGTGATCGAACAGCGTTACCCGGCCGCGGGCGATGCCAACGTGCAGGTGAAGCTGGGCGTGATCTCGCCGGCGGAACAGGCGCAGACCCAGTGGATCGAC   840
V  Y  A  D  R  T  D  V  I  E  Q  R  Y  P  A  A  G  D  A  N  V  Q  V  K  L  G  V  I  S  P  A  E  Q  A  Q  T  Q  W  I  D
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L  G  K  E  Q  D  I  Y  L  A  R  V  N  W  R  D  P  Q  H  L  S  F  Q  R  Q  S  R  D  Q  K  K  L  D  L  V  E  V  T  L  A
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S  N  Q  Q  R  V  L  A  H  E  T  S  P  T  W  V  P  L  H  N  S  L  R  F  L  D  D  G  S  I  L  W  S  S  E  R  T  G  F  Q
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D  S  W  S  N  N  S  T  P  P  Q  I  E  L  F  R  A  N  G  E  K  I  A  T  L  V  E  N  D  L  A  D  P  K  H  P  Y  A  R  Y
CGCGAGGCGCAGCGCCCGGTCGAATTCGGCACGCTGACTGCCGCCGACGGCAAGACCCCGCTGAACTACAGCGTGATCAAGCCGGCAGGATTCGATCCGGCCAAGCGCTACCCGGTGGCG  1560
R  E  A  Q  R  P  V  E  F  G  T  L  T  A  A  D  G  K  T  P  L  N  Y  S  V  I  K  P  A  G  F  D  P  A  K  R  Y  P  V  A
GTGTACGTGTATGGCGGCCCGGCCAGCCAGACCGTCACCGACAGCTGGCCCGGCCGTGGCGACCATCTGTTCAACCAGTACCTGGCCCAGCAGGGCTATGTGGTGTTCTCGCTGGACAAC  1680
V  Y  V  Y  G  G  P  A  S  Q  T  V  T  D  S  W  P  G  R  G  D  H  L  F  N  Q  Y  L  A  Q  Q  G  Y  V  V  F  S  L  D  N
CGCGGCACCCCGCGTCGTGGCCGTGACTTCGGCGGTGCGCTGTATGGCAAGCAGGGTACGGTGGAAGTGGCCGACCAGCTGCGTGGCGTGGTTGAAGCAGCAGCCGTGGGTGGAC  1800
R  G  T  P  R  R  G  R  D  F  G  G  A  L  Y  G  K  Q  G  T  V  E  V  A  D  Q  L  R  G  V  A  W  L  K  Q  Q  P  W  V  D
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P  A  R  I  G  V  Q  G  W  S  N  G  G  Y  M  T  L  M  L  L  A  K  A  S  D  S  Y  A  C  G  V  A  G  A  P  V  T  D  W  G
CTGTACGACAGCCACTACACCGAGCGCTACATGGACCTGCCGGCGCGCAACGATGCCGGCTACCGCGAAGCACGCGTGCTGACCCACATCGAGGGCCTGCGATCGCCGCTGCTGCTGATC  2040
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CACGGCATGGCTGACGACAACGTGCTGTTCACCAATTCGACCAGCCTGATGAGCGCGCTGCAGAAGCGTGGCCAGCCGTTTGAACTGATGACCTATCCGGGCGCCAAGCACGGTCTGTCC  2160
H  G  M  A  D  D  N  V  L  F  T  N  S  T  S  L  M  S  A  L  Q  K  R  G  Q  P  F  E  L  M  T  Y  P  G  A  K  H  G  L  S
GGCGCCGACGCACTGCATCGCTACCGCGTCGCCGAAGCCTTCCTGGGACGTTGCCTGAAGCCTTGATCATCATCCGGGAAGGAGCCCGCCCATGACCCTGCCGCCGCCGATTCCTGCTCC  2280
G  A  D  A  L  H  R  Y  R  V  A  E  A  F  L  G  R  C  L  K  P  *
CTCCCACCTGCGATCTGGCTGGTGGTGCCGGCATTGGCGCTGGGCGATGCCATTGGCGGTGCTGCTGTTCCTGGTCGCACTGGGCGGCGCCATCGGCTGGAGCGTGCTGCGCTGGACCGA  2400
GGCCGCACGACAGTGCGCCGATGCGCGAGGCGATGCGTCGCGCCGGCTGCAGCGTCGAACTGGTGCAGGTGTTCGGCGAACCGCTGCAG                              2489
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Fig. 2. **Nucleotide sequence of the *X. maltophilia* dipeptidyl peptidase IV gene and its deduced amino acid sequence.** The double underline indicates the putative transmembrane domain as deduced from the hydropathy profile.

in 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA. The enzyme in the active fractions was precipitated by salting out with ammonium sulfate, and the precipitate was dissolved in a small volume of 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, then desalted by gel filtration on a Sephadex G-25 column equilibrated with the same buffer. The enzyme active fractions were combined and applied to a column (6×15 cm) of DEAE-Toyopearl 650C equilibrated with the above buffer. The enzyme was not adsorbed, but passed through the column, leaving a large fraction of the unwanted proteins on the column. Active fractions were combined and concentrated by ultrafiltration using an Amicon apparatus (PM-10), and applied to a hydroxyapatite column (2×20 cm) equilibrated with 10 mM phosphate buffer (pH 8.0). The enzyme was eluted with a linear gradient of potassium phosphate buffer (10-500 mM). Active fractions were combined, concentrated by ultrafiltration using an Amicon apparatus (PM-10), and dialyzed against 10 mM potassium phosphate buffer (pH 8.0).

Crystallization—Crystals of DP IV were grown at 20°C using the hanging drop vapor diffusion technique. Drops of 10 μl were prepared by mixing 5 μl of the protein solution (20 mg/ml) with 5 μl of the reservoir solution: 0.1 M Na cacodylate pH 6.5, 0.2 M Mg acetate, and 20% PEG 6000.

RESULTS

Cloning of the Dipeptidyl Peptidase IV Gene—Among approximately 1,000 transformants screened, one showed enzyme activity and was found to be harboring a plasmid with a 3.2 kbp insert in the *Bam*HI site. This recombinant plasmid was designated pXDP. The restriction map of the insert is shown in Fig. 1.

The association between the cloned gene and DP IV was further confirmed by Southern hybridization, using the 3.2 kbp fragment as a probe. Only one band of the *Bam*HI-digested *X. maltophilia* chromosomal DNA hybridized with the labeled probe, and its size was approximately the same as that of the insert contained in pXDP. This result not only confirms the identity of the cloned gene, but also shows that DP IV exists as a single gene in *X. maltophilia* (data not shown).

Nucleotide Sequence—The nucleotide sequence of the insert in pXDP was determined. Within this sequence, there was an open reading frame of 2,223 bp, beginning with an ATG methionine codon (Fig. 2). The protein deduced from the nucleotide sequence was composed of 741 amino acid residues, with a molecular weight of 82,080. The sequence starts with a transmembrane region which has a hydrophobic core domain, as deduced from the hydropathy profile.

Purification and Crystallization of the Expressed Enzyme—The purification procedure is summarized in Table I. The enzyme was purified 1,030-fold, with an activity recovery of 50% from cell-free extracts. By SDS-PAGE, the final preparation showed a single band corresponding to a molecular weight of 83,000 (Fig. 3), which agreed well with that calculated from the deduced amino acid sequence. The enzyme was crystallized by the hanging drop vapor diffusion method using PEG 6000 as a precipitant. A

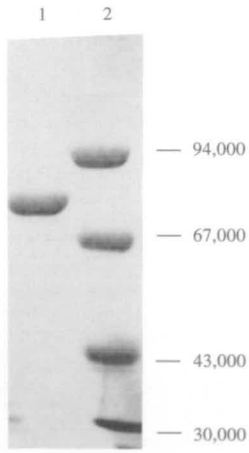

Fig. 3. **SDS-PAGE of the purified enzyme preparation.** SDS-PAGE was run on 7.5% polyacrylamide gels, which were subsequently stained with Coomassie Brilliant Blue R-250. Lane 1, purified enzyme; lane 2, marker proteins.

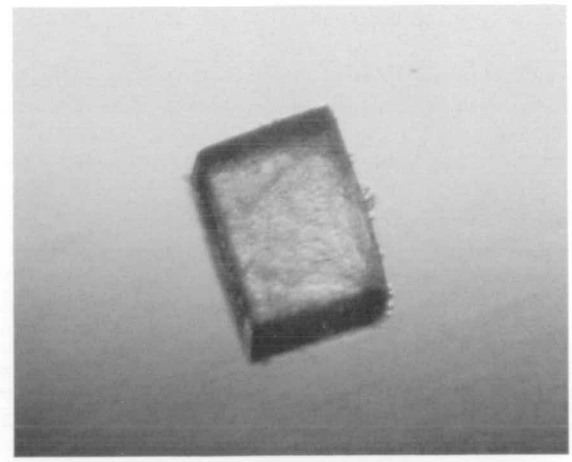

Fig. 4. **Photomicrograph of the crystal of DP IV.** The crystal was produced in hanging drop, by mixing 5 μl of protein solution (20 mg/ml) and 5 μl of reservoir buffer containing 20% PEG 6000 (pH 6.5).

TABLE I. **Purification of the dipeptidyl peptidase IV from *E. coli* DH 1/pXDP.**

Purification step	Total protein (mg)	Total activity (units)	Recovery (%)	Specific activity (units/mg)	Purification ratio (fold)
Cell-free extract	36,000	12,400	100	0.34	1
Supernatant after 40% $(NH_4)_2SO_4$ precipitation	12,800	9,550	77.0	0.75	2.2
Toyopearl HW65C	707	8,210	66.2	11.6	34.1
1st DEAE-Toyopearl	27.6	6,340	51.1	230	676
2nd DEAE-Toyopearl	22.6	6,300	50.8	280	824
Hydroxyapatite	17.7	6,200	50.0	350	1,030

TABLE II. **Substrate specificity of DP IV from *X. maltophilia*.**

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1} \cdot mM^{-1}$)
Gly-Pro-2NNap	0.15	124	827
Ala-Pro-pNA	0.33	125	379
Ser-Pro-pNA	0.39	127	326
Lys-Pro-pNA	0.44	131	298
His-Pro-pNA	0.33	45.0	136
Glu-Pro-pNA	0.16	53.6	335

2NNap, β-naphthylamide; pNA, *p*-nitroanilide. No hydrolysis: z-Gly-Pro-2NNap, Pro-pNA.

TABLE III. **Effect of various inhibitors on DP IV activity.**

Inhibitors	Conc. (mM)	Remaining activity (%)
None		100
EDTA	5.0	124
o-Phenanthroline	1.0	90.7
Iodoacetamide	1.0	91.1
PCMB	1.0	96.7
DFP	1.0	0
PMSF	1.0	50.2
	5.0	3.7

The enzyme was preincubated at 37°C (pH 8.0) for 30 min.

TABLE IV. **Physicochemical properties of DP IV.**

Optimum pH	8.5
pH stability[a]	7.0–9.0
Optimum temperature	50°C
Thermal stability[b]	45°C
Isoelectric point	6.7
Molecular weight	83,000 (SDS-PAGE)
	165,000 (gel filtration)

[a]37°C for 60 min. [b]pH 8.0 for 60 min.

rectangular prismatic crystal, shown in Fig. 4, was observed.

Enzymatic and Physicochemical Properties—The purified enzyme catalyzed the hydrolysis of peptide derivatives which contain proline in the position penultimate to the free amino terminus (Table II). The enzyme also split the Ala-X bond when alanine was located at the same position (data not shown). The enzyme was inert toward peptides in which the terminal amino acid was protected.

The enzyme was markedly inhibited by DFP but was hardly affected by SH reagents or metal chelators (Table III).

Some properties of the purified enzyme are summarized in Table IV. The isoelectric point was 6.7, and the molecular weight was estimated to be 83,000 by SDS-PAGE, and 165,000 by gel filtration, suggesting that the enzyme exists as a dimer.

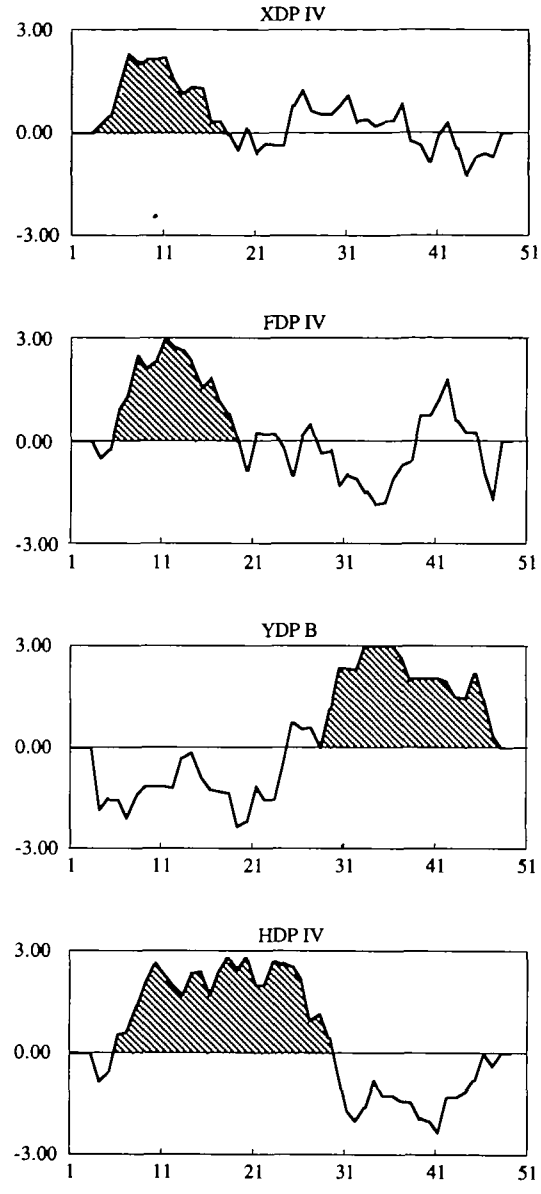

Fig. 5. **Hydropathy profiles of the transmembrane domain of four enzymes.** Hydrophobicity values of 50 amino acids from the N-termini, obtained according to the methods of Kyte and Doolittle (*38*), have been plotted with respect to their positions in the amino acid sequences. The window used in the scanning was 7 amino acids. Line segments above and below the horizontal axis indicate hydrophobic and hydrophilic portions, respectively. The shadowed region indicates the putative transmembrane domain. XDPIV, *X. maltophilia* DP IV; FDPIV, *F. meningosepticum* DP IV (*30*); YDPB, yeast DP B (*39*); HDPIV, human DP IV (*40*).

DISCUSSION

Dipeptidyl peptidase IV from *X. maltophilia* was cloned and expressed in *E. coli*. The expressed protein was purified and crystallized. In a previous paper, we reported on prolidase (*27*) and prolyl carboxypeptidase (*28*) from *Xanthomonas*. Szwajcerdey *et al.* (*29*) reported on prolyl endopeptidase, also from *Xanthomonas* sp. Similarly, *F. meningosepticum* produces prolyl endopeptidase (*16*), DP IV (*30*), and prolyl aminopeptidase (unpublished data). Both *Xanthomonas* and *Flavobacterium* produce many kinds of proline-specific peptidases, though the biological significance of these enzymes is not clear.

The open reading frame detected in the DP IV gene by nucleotide sequence analysis, encodes a protein consisting of 741 amino acid residues with a molecular weight of 82,080. The molecular weight of the purified enzyme was estimated to be 83,000 and 165,000 by SDS-PAGE and gel filtration, respectively, suggesting that the enzyme is a dimer, as are the other reported enzymes. The optimum temperature for the enzyme activity was 50°C, similar to that of the *F. meningosepticum* enzyme, while that of

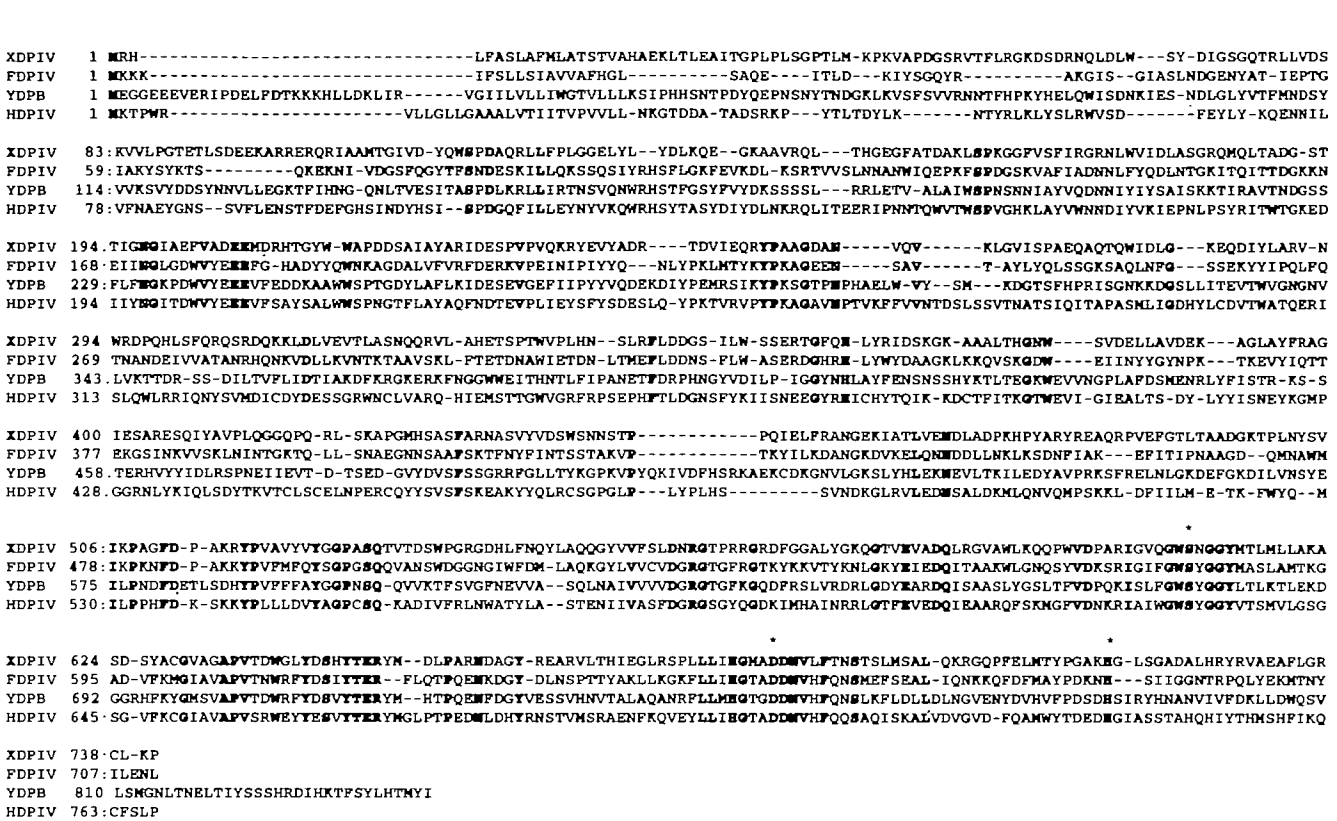

Fig. 6. **Comparison of the amino acid sequences of the dipeptidyl peptidases.** The asterisks indicate the catalytic triad residues. XDPIV, *X. maltophilia* DP IV; FDPIV, *F. meningosepticum* DP IV; YDPB, yeast DP B; HDPIV, human DP IV.

mammalian enzymes is around 60°C (*5*). The enzyme was completely inhibited by DFP and slightly inhibited by PMSF, as are the enzymes from mammals. Substrate specificity was also similar to that of the other enzymes. The enzyme catalyzed hydrolysis of the Pro-X bond, with no influence of the amino acid at the P2 position.

To compare the expressed enzyme with the wild-type enzyme, the enzyme was purified from *X. maltophilia*. Purification of the wild-type enzyme was done by using the same procedure as for the expressed enzyme, but the preparation contained some contaminants as judged by SDS-PAGE. Using this partially purified enzyme, the enzymatic and physicochemical properties of the wild-type enzyme were determined. The wild-type enzyme has the same optimum pH and temperature, as well as pH and thermal stability, as the expressed enzyme. The wild-type enzyme was also markedly inhibited by DFP and PMSF at 1 mM and 5 mM, respectively, but was hardly affected by SH reagents and metal chelators (data not shown). These findings and the results of hybridization with the enzyme gene and chromosome suggested that the enzyme expressed in *E. coli* is derived from *X. maltophilia*.

Since DP IV is a membrane-bound enzyme, many previously applied methods are available for its extraction. Bovine and lamb kidney enzymes have been extracted by autolysis (*4*), and papain digestion (*31*), while the human placental enzyme has been extracted by combined treatment with 10% butanol followed by 1% Nonidet P40 (*32*). The porcine pancreas enzyme has been extracted from acetone powder (*33*), while *F. meningosepticum* enzyme has been extracted by sonication in the presence of 1% sodium cholate (*5*). The DP IV enzyme from *X. maltophilia*, however, could be extracted under rather mild conditions with 0.1% SDS. Figure 5 shows the hydropathy profiles of the putative transmembrane domain of enzymes from *X. maltophilia*, *F. meningosepticum*, yeast, and humans. The hydrophobicity of the enzyme from *X. maltophilia* was lower than that of the others. No positively charged amino acid was present in front of the transmembrane domain in *X. maltophilia* DP IV. This seems to make it easier to extract the enzyme from the membrane.

The amino acid sequence was 30% homologous to that of *F. meningosepticum* DP IV, and 25% homologous to those of human DP IV and yeast DP B (Fig. 6). Although the overall homology level is low, the C-terminal part involving the active residues (Ser, Asp, and His) is well conserved. When the amino acid sequence of *X. maltophilia* DP IV was compared with that of *Lactococcus lactis* X-prolyl dipeptidylaminopeptidase (XPDA), which has the same substrate specificity as DP IV, only minimal similarity around the active serine residue (G-X-S-X-X-G) was observed.

DP IV is included in the prolyl oligopeptidase family, based on comparisons of amino acid sequences (*16–18*). This family, which includes prolyl endopeptidase (*16*), protease II (*19*), and acylamino acid releasing enzyme (*34*), is not related to the well-known chymotrypsin and subtilisin families. Although the homology among the enzymes of this family is 15–30%, the homology level is greater at the C-termini involving the catalytic triad (Ser, Asp, and His). The sequential order of this catalytic triad in the prolyl oligopeptidase family (Ser-Asp-His) is different from those in the chymotrypsin (His-Asp-Ser) and sub-

tilisin (Asp-His-Ser) families.

The sequential order of the catalytic triad of serine carboxypeptidase II (*20*), lipase (*21*), and acetylcholinesterase (*22*), is the same as that of members of the prolyl oligopeptidase family. However, the amino acid sequence homology of these enzymes is low. Recently, it was confirmed that acetylcholinesterase (*22*), lipase (*21*), serine carboxypeptidase II (*20*), dienelactone hydrolase (*35*), and haloalkane dehalogenase (*36*), all of whose structures have already been determined, share the same α/β-hydrolase fold (*24*). Based on the similarity of amino acid sequence (unpublished data), prolyl aminopeptidase also seems to be a member of this α/β-hydrolase fold family.

These findings indicate that the three-dimensional structure of DP IV may have a similar topology to an α/β-hydrolase fold. In order to clarify this, we crystallized the enzyme using the hanging drop vapor diffusion method. A preliminary X-ray analysis of XPDA from *L. lactis* has recently been reported (*37*). However, the amino acid sequence of DP IV from *X. maltophilia* is closer to those of mammalian enzymes than to XPDA, so an X-ray crystallographic analysis of this DP IV is likely to be of interest.

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