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Dipeptidyl Peptidase IV from Human Serum: Purification, Characterization, and N-Terminal Amino Acid Sequence

Sachiko Iwaki-Egawa,*,[1] Yasuhiro Watanabe,* Yasuoki Kikuya,* and Yukio Fujimoto†

*Department of Clinical Biochemistry and †Department of Biochemistry, Hokkaido College of Pharmacy, 7-1 Katsuraoka-cho, Otaru, Hokkaido 047-0264

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Dipeptidyl peptidase IV (DPP IV) in normal human serum was purified 14,400-fold with a 25% yield to homogeneity. The molecular weight of the purified enzyme was approximately 110,000 on SDS-PAGE, almost the same as that of human kidney membrane-bound DPP IV. No difference was found between the two enzymes enzymologically and immunologically, either in substrate specificity, susceptibility to inhibitors, or cross-reactivity with an anti-rat kidney DPP IV antibody, or in their ability to bind adenosine deaminase. However, the N-terminal amino acid sequence of serum DPP IV lacked the transmembrane domain of the membrane-bound enzyme and started at the 39th position, serine, from the N-terminus predicted from the cDNA nucleotide sequence. These results suggest that membrane-bound DPP IV loses its transmembrane domain upon release into the serum, and that its structure on the plasma membrane is not required for its binding to adenosine deaminase.

Key words: adenosine deaminase, dipeptidyl peptidase IV, human serum, kidney, N-terminal amino acid sequence.

In recent years, special interest has been paid to the aminopeptidases localized on the plasma membrane of immune cells. These aminopeptidases are able to inactivate peptides, and most of them have a common structure which is called a type II integral membrane protein, *e.g.*, aminopeptidase A (APA/BP-1/6C3; EC 3.4.11.7) (*1, 2*), cystine aminopeptidase (oxytocinase) (CAP; EC 3.4.11.3) (*3, 4*), neutral endopeptidase (NEP/CD10; EC 3.4.24.11) (*5*), aminopeptidase N (APN/CD13; EC 3.4.11.2) (*6*), and dipeptidyl peptidase IV (DPP IV/CD26; EC 3.4.14.5) (*7-10*). The structure of the type II integral membrane protein consists of a single hydrophobic N-terminal domain, a transmembrane region, and the C-terminal portion containing a catalytic site.

One of these molecules, DPP IV can release N-terminal dipetides, preferentially cleaving prolyl or alanyl bonds (*11, 12*). By virtue of its predominant localization in the brush border of small intestine and kidney proximal tubules, it was initially suggested that DPP IV participates in the metabolism and the uptake of proline-containing peptides in these tissues (*13*). Recent studies have also revealed that DPP IV is a leukocyte differentiation antigen, CD26, which is expressed on a subpopulation of human T cells (*14, 15*). DPP IV/CD26 transduces differentiation and activation signals in T cells (*16-18*) and has been associated with the susceptibility of a T cell line to infection with monocytotropic HIV-1 (*19*). Oravecz, T. *et al.* identified the chemokines RANTES (regulated on activation, normal T cell expressed and secreted), interferon-γ-inducible protein monocyte chemotactic protein (MCP)-2, eotaxin, and IP (interferon-γ-inducible protein)-10 as the first natural CD26 substrates with immune function (*20*). Moreover, it has been conclusively demonstrated that in human, DPP IV/CD26 is equivalent to adenosine deaminase (ADA; EC 3.5.4.4)-binding protein (*21, 22*). An inherited deficiency of ADA causes severe combined immunodeficiency disease (SCID), which is characterized by the absence of functional T and B cells in affected individuals (*23*).

DPP IV functions as a membrane-bound peptidase on the cell surface as described above. On the other hand, DPP IV activity is also present in body fluids such as serum (*24*) and seminal plasma (*25*). The DPP IV activity in serum is found to be elevated in patients with hepatitis and hepatobiliary diseases, but decreased in patients with gastric cancer, pancreatic cancer, systemic lupus erythematosus, and rheumatoid arthritis (*26-28*).

Because of the hydrophobic domain which fixes the molecule to the membrane, it is necessary to use detergent-containing solution to solubilize the entire membrane-bound DPP IV from the membrane. We reasoned, therefore, that the deletion of the hydrophobic domain might be essential for the existence of DPP IV in serum. In this study, we describe the purification of DPP IV from human serum and its characterization, including its N-terminal amino acid sequence analysis.

[1] To whom correspondence should be addressed. Phone: +81-134-62-5111, FAX: +81-134-62-5161, E-mail: sachiko@hokuyakudai.ac.jp

Abbreviations: ADA, adenosine deaminase; APN, alanine aminopeptidase N; CAP, cystine aminopeptidase; DFP, diisopropyl fluorophosphate; DPP IV, dipeptidyl peptidase IV; HRPO, horseradish peroxidase; pNA, p-nitroanilide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Materials—Human serum was provided by the Hokkaido Red Cross Blood Center (Sapporo). Human kidney was kindly provided by the Department of Anatomy, School of Medicine, Sapporo Medical University (Sapporo), and exhibited no pathological abnormalities on a histological examination. Both were stored at −80°C until use.

Chromatographic resins were purchased from the following sources: DEAE-Cellulose DE-52 (Whatman, Maidstone, England); DEAE-Cellulofine A800 (Seikagaku, Tokyo); Sephacryl S-300 HR and CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden); butyl-Toyopearl S (Tosoh, Tokyo); anti-rat kidney DPP IV-Sepharose 4B (*29*).

The following compounds were used as substrates and inhibitors: Gly-Pro-pNA, Val-Ala-pNA, β-casomorphine-5, substance P, diprotin-A (Peptide Institute, Osaka); Ala-Ala-pNA, Pro-pNA (Sigma Chemicals, St. Louis, MO, USA); Ala-Pro-pNA (Bachem Feinchemikalien AG, Bubendorf, Switzerland); Z-Gly-Pro-pNA (Cambridge Research Biochemicals, Cambridgeshire, England); DFP (Wako Pure Chemical Industries, Osaka).

Recombinant *N*-glycanase was purchased from Genzyme (Cambridge, MA, USA). HRPO-conjugated Protein A, ECL detection kit, and Hyperfilm-^{3}H were purchased from Amersham (Cleveland, OH, USA). Calf intestinal mucosa ADA was purchased from Sigma Chemicals (St. Louis, MO, USA). Alkaline phosphatase conjugated anti-rabbit IgG (H+L) was purchased from Jackson Immuno Research Laboratories (West Grove, PA, USA).

All other reagents were of the highest quality available from commercial sources.

Assay of Proteolytic Activity—Enzyme activity was assayed by liberation of pNA in an assay mixture containing 1 mM substrate, 20 mM Tris-HCl (pH 8.0), and enzyme in a total volume of 0.25 ml. After incubation for 30 min at 37°C, the reaction was stopped by adding 0.75 ml of 20% acetic acid, then the increase in absorbance at 410 nm was measured.

Protein Assay—Protein concentration was determined by measuring the absorbance of the sample solution at 280 nm or by use of a BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

Polyacrylamide Gel Electrophoresis—SDS-PAGE was carried out using 7.5% polyacrylamide gels according to the method of Laemmli (*30*).

Determination of N-Terminal Amino Acid Sequence of DPP IV—Purified DPP IV was electrophoresed on a 7.5% SDS-PAGE gel, electrotransferred to polyvinylidene difluoride (PVDF) membranes (Pro Blott; Applied Biosystems, Foster City, CA, USA), and stained with 0.1% Ponceau S. The areas corresponding to DPP IV band were cut off. The destained PVDF membranes thus obtained were subjected to an Applied Biosystems model 477A Protein Sequencer.

Preparation of Antibodies—To prepare antiserum against human membrane-bound kidney DPP IV, a peptide fragment consisting of 16 amino acid residues (MKTPWKVLLGLLGAAA) from the N-terminus of kidney DPP IV was synthesized by the Fmoc-polyamide solid phase method. A Cys residue was added to the C-terminus of the peptide to allow coupling to keyhole lympet hemocyanin with maleimido-benzoyl-*N*-hydroxysuccinimide ester. A KBL: JW rabbit was immunized by subcutaneous injection of Freund's complete adjuvant containing 1.25 mg of conjugate. Booster injections of 1.25 mg of the conjugate were performed three times at 3-week intervals. Serum was collected 73 days after the first injection. Immunoglobulins in the anti-serum were purified as described previously (*31*).

Rat kidney DPP IV was purified and a rabbit IgG anti-rat kidney DPP IV antibody was raised as described before (*32*).

A specific rabbit IgG anti-calf ADA antibody was raised as described before (*33*).

Purification of Human Serum DPP IV—All purification steps were performed at 4°C.

Step 1. DEAE-Cellulose DE-52 column chromatography: Human serum (200 ml) was dialyzed against 10 mM Tris-HCl buffer, pH 8.0 (buffer A). The dialyzate was centrifuged at 10,000×*g* for 10 min to remove the insoluble materials. The supernatant was used in the subsequent steps. The supernatant was applied to a DEAE-Cellulose DE-52 column (2.5×40 cm) previously equilibrated with buffer A. The column was washed with the equilibration buffer, then developed with 2 liters of a linear gradient of NaCl (0-0.3 M). Fractions containing DPP IV activity were pooled and dialyzed against buffer A.

Step 2. DEAE-Cellulofine A800 column chromatography: The dialyzed fractions were rechromatographed on a second anion-exchange column (DEAE-Cellulofine A800, 2.5×35 cm) equilibrated with buffer A. The column was washed, then developed with 1 liter of a linear gradient of NaCl (0-0.2 M). The active fractions were pooled and concentrated using 10-kDa cut-off centrifugal concentrators (Centriprep 10; Amicon, Beverly, MA, USA).

Step 3. Sephacryl S-300 HR column chromatography: The concentrated sample was applied to a Sephacryl S-300 HR column (2.5×90 cm) equilibrated with buffer A containing 0.1 M NaCl. The column was developed with the same buffer and the active fractions were pooled. The solution was adjusted to 40% saturation with ammonium sulfate.

Step 4. Butyl-Toyopearl S column chromatography: The solution was applied to a butyl-Toyopearl S column (1.5×7 cm) equilibrated with 40% ammonium sulfate-saturated buffer A. The column was washed with the same buffer, then the bound proteins were eluted with 200 ml of a linear gradient of ammonium sulfate, 40-0%. The active fractions were dialyzed against buffer A. This preparation was used in subsequent experiments.

Purification of Human Membrane-Bound Kidney DPP IV—An intact membrane-bound kidney DPP IV was purified as follows. All purification steps were performed at 4°C unless otherwise stated. Supernatants of the microsomes of human kidney were prepared as described before (*34*). DPP IV was purified from the supernatant by passing it through a rabbit IgG anti-rat kidney DPP IV Sepharose 4B column (1.5×8 cm) equilibrated with buffer A containing 0.05% Triton X-100 and 0.15 M NaCl. After washing the column with the same buffer, the bound protein was eluted with 50 mM diethylamine-HCl (pH 11.0) containing 0.05% Triton X-100 at room temperature. Immediately, the active fractions were pooled and dialyzed against buffer

A containing 0.05% Triton X-100.

Western Blotting—Purified DPP IV was electrophoresed on a 7.5% SDS-PAGE gel and electrotransferred to PVDF membranes. After the electroblotting, the membranes were treated with rabbit IgG anti-rat kidney antibody (10 μg/ml) or rabbit IgG anti-peptide antibody (25 μg/ml). HRPO-conjugated Protein A was used as a second antibody. The enhanced chemiluminescence (ECL) system (Amersham) was used for detection. The membranes were subsequently exposed to Hyperfilm-^{3}H autoradiography film and processed.

N-Glycanase Treatment—An equal volume of 40 mM NaH_2PO_4-Na_2HPO_4 (pH 8.0) was added to purified DPP IV (10 μl, 1.5 μg protein), and the mixture was boiled in the presence of SDS and β-mercaptoethanol for 5 min to denature the protein. Then 7.5% Nonidet P-40 (5 μl) and 250 U/ml recombinant *N*-glycanase (2 μl) were added, and the mixture was incubated for 21 h at 37°C.

DPP IV Binding to Adenosine Deaminase—Samples of serum DPP IV (72.5 mU) and kidney DPP IV (52 mU) were incubated for 30 min at 37°C in the presence or absence of calf intestinal ADA (ADA-S, 1 μg) in a total volume of 20 μl. The reaction mixtures were run on a 4-20% non-SDS-PAGE gel. Proteins on the gel were detected by Coomassie Brilliant Blue staining and by Western blotting using a specific rabbit IgG anti-calf ADA antibody (10 μg/ml). Alkaline phosphatase conjugated anti-rabbit IgG (H+L) was used as a second antibody and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Wako Pure Chemical Industries) was used as substrate.

RESULTS AND DISCUSSION

Purification of Human Serum DPP IV—Human serum DPP IV was isolated by sequential purification using ion exchange chromatography (DEAE-Cellulose DE-52 and DEAE-Cellulofine A-800), gel filtration on a Sephacryl S-300 HR column, and hydrophobic chromatography on a butyl-Toyopearl 650S column. The purification steps for DPP IV are summarized in Table I. Serum DPP IV was purified 14,400-fold with a 25% yield over serum. Its specific activity was 19.1 μmol/min/mg protein with Gly-Pro-pNA as the substrate. The purified enzyme migrated as a single band on SDS-PAGE under reducing conditions (Fig. 1). The molecular mass of the enzyme was estimated to be 110 kDa, similar to that of the human membrane-bound kidney DPP IV. The purified enzyme also showed the same molecular weight band under non-reducing conditions (data not shown). We have previously reported that our anti-rat kidney DPP IV antibody recognizes a 60-kDa fragment of rat kidney DPP IV cut off at the 281st residue from the N-terminus (*34*). DPP IVs from serum and kidney showed cross-reactivity to the anti-rat kidney DPP IV antibody in Western blotting, and their molecular sizes were identical (data not shown).

Comparison of Enzymatic Properties of Kidney and Serum DPP IVs—The relative rates of hydrolysis of various pNA substrates by the purified enzyme preparations are shown in Table II. Kidney and serum DPP IVs showed almost the same substrates specificities. To characterize the specificities and action of the enzymes further, the hydrolysis of some biologically active peptides was investigated. Substance P and β-casomorphine-5 were used. Both peptides are known to contain Pro residues at the second and the fourth positions from the N-termini, and the N-terminal dipeptide and subsequent dipeptide from substance P and β-casomorphine-5 are hydrolyzed by DPP IV (*35*). No difference in the hydrolysis of these compounds was found between the two DPP IVs (data not shown).

Kidney DPP IV is a serine protease and an aminopeptidase which can be inhibited by DFP (*25*) and diprotin-A (*36*), respectively. We examined the effects of DFP and diprotin-A on DPP IVs from kidney and serum. Both DPP IVs were inhibited by DFP and diprotin-A in the same manner. ID_{50} of DFP for kidney and serum DPP IVs was 0.55 and 0.50 mM, respectively, and ID_{50} of diprotin-A for kidney and serum DPP IVs was the same (20 μM). Therefore, we can confirm that serum DPP IV is a serine proteinase.

N-Glycanase Treatment of DPP IVs—DPP IV is regularly present as a non-covalently linked homodimeric glycoprotein, consisting of two *N*-glycosylated subunits of 110 kDa (*8*). So we investigated the pattern of glycosylation of the membrane-bound and serum forms. As shown in Fig. 2, the deglycosylated backbone of serum DPP IV is slightly smaller than that of kidney DPP IV. This result demon-

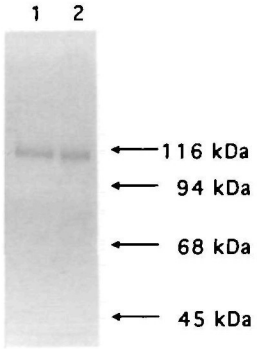

Fig. 1. **SDS-PAGE of DPP IVs from human normal serum and kidney.** Samples were subjected to SDS-PAGE (7.5% gel) under reducing conditions, followed by protein staining (Coomassie Brilliant Blue R-250). Marker proteins used are β-galactosidase (116 kDa), phosphorylase *a* (94 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa). Lane 1: serum; lane 2: kidney.

TABLE I. **Purification of DPP IV from normal human serum.**

Step	Total protein (mg)	Total activity[a] (units)	Specific activity (mU/mg protein)	Yield (%)	Purification (-fold)
Human serum	12,600	16.8	1.33	100	1
DEAE-Cellulose DE-52	2,490	8.98	3.61	53.5	2.71
DEAE-Cellulofine A-800	1,560	8.68	5.56	51.7	4.18
Sephacryl S-300 HR	102	7.05	68.8	42.0	52.0
Butyl-Toyopearl 650S	0.22	4.20	19,100	25.0	14,400

[a]One unit (U) is defined as the amount of enzyme cleaving 1 μmol of Gly-Pro-pNA/min.

strates that there is a difference in the primary structure of the DPP IVs.

Comparison of the N-Terminal Amino Acid Sequences— To investigate the primary structure of DPP IVs, the N-terminal amino acid sequences of membrane-bound kidney and serum DPP IVs were determined and compared with the amino acid sequence predicted from the cDNA nucleotide sequence of human liver DPP IV (*8*) (Fig. 3). The entire amino acid sequence of human kidney DPP IV including the transmembrane site has not yet been reported. However, we revealed that the N-terminal sequence of human kidney membrane-bound DPP IV was completely identical to the predicted structure. On the other hand, the N-terminal sequence of serum DPP IV was identified in a sequence starting at the 39th position, serine, from the N-terminus. These results suggest that membrane-bound DPP IV loses its transmembrane domain upon release into the serum.

To confirm the loss of the transmembrane domain, the cross-reactivity of serum DPP IV with an anti-peptide antibody against the N-terminus of kidney DPP IV was examined. A peptide fragment consisting of 16 amino acid residues (MKTPWKVLLGLLGAAA) from the N-terminus of kidney DPP IV was synthesized using the Fmoc-polyamide solid-phase method, and a polyclonal antibody was raised in rabbit. Kidney membrane-bound DPP IV cross-reacted with the anti-peptide antibody, but serum DPP IV did not (Fig. 4). This result supports the N-terminal amino acid sequence analysis of serum DPP IV as lacking the transmembrane domain.

Our previous studies have shown that APN and CAP in sera from pregnant women are differently processed derivatives of membrane-bound APN and CAP, respectively (*37, 38*). Furthermore, our recent result indicates that APN in normal human serum is a differently processed derivative from the one in maternal serum (*39*). DPP IV, like APN and CAP, is a type II integral membrane aminopeptidase. We can, therefore, propose with certainty that the type II integral membrane aminopeptidases can be released from the membrane to the blood circulation by differential limited proteolysis, though the mechanism of release remains unclear.

While this study was in progress, Duke-Cohan *et al.* reported that a novel form of DPP IV was found in human serum that was not a cleavage product of CD26/DPP IV (*40*). Their DPP IV existed as a monomer of 175 kDa and the N-terminal sequences of its tryptic digest have no homology with CD26/DPP IV. They also reported finding a novel activated T cell antigen (DPPT-L), and that the 175-kDa DPP IV is similar to DPPT-L released from activated T cells (*41*). We think the inconsistency between their findings and our data might have resulted from their missing the real serum DPP IV during their purification processes. Our conclusion indicates that serum DPP IV is a cleavage product of 110-kDa membrane-bound DPP IV. Furthermore, it seems that what Duke-Cohan *et al.* call 175-kDa DPP IV is quite different from real serum DPP IV

TABLE II. **Comparison of the substrate specifity of DPP IVs from kidney and serum.**

Substrate	Relative activity (%)	
	Kidney	Serum
Gly-Pro-pNA	100	100
Ala-Pro-pNA	66.4	69.1
Val-Ala-pNA	16.9	20.5
Ala-Ala-pNA	14.2	11.5
Z-Gly-Pro-pNA	0	0
Pro-pNA	0	0

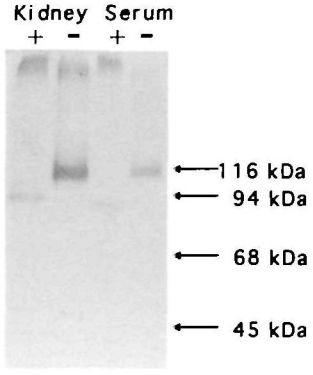

Fig. 2. ***N*-Glycanase digestion of DPP IVs from kidney and serum.** The proteins were incubated overnight at 37°C in the presence (+) or absence (−) of *N*-glycanase as described under "MATERIALS AND METHODS." The digested proteins were subjected to SDS-PAGE (7.5% gel), followed by Western blotting with a rabbit anti-rat kidney DPP IV antibody. Marker proteins used are β-galactosidase (116 kDa), phosphorylase *a* (94 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa).

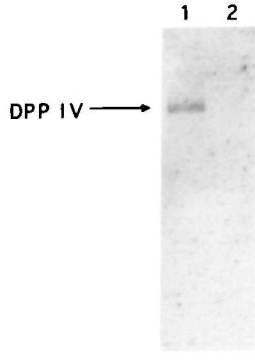

Fig. 4. **Western blotting of DPP IVs from kidney and serum with a rabbit anti-peptide antibody.** Samples were subjected to SDS-PAGE (7.5% gel), followed by Western blotting with a rabbit anti-peptide antibody. Lane 1: kidney; lane 2: serum.

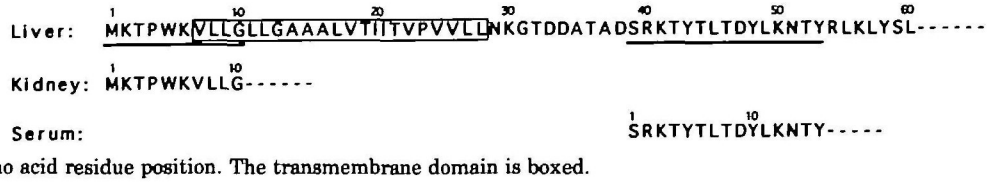

Fig. 3. **Sequence comparison of the human membrane-bound kidney and serum DPP IVs with liver DPP IV.** Sequences of the human liver (*8*), kidney, and serum DPP IVs are aligned: —, identical amino acids; numbers, amino acid residue position. The transmembrane domain is boxed.

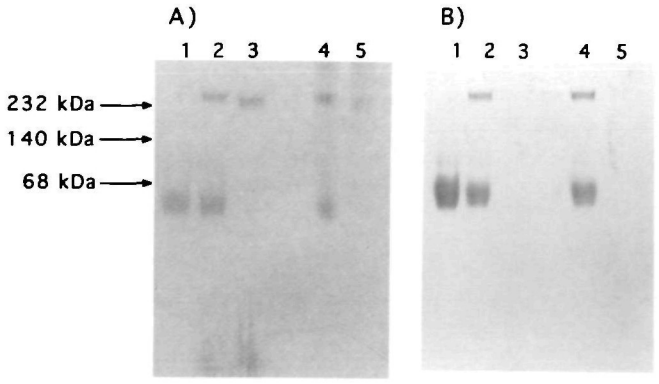

Fig. 5. **Binding of serum and kidney DPP IVs to ADA.** Samples were subjected to non-SDS-PAGE (4-20% gel), followed by Coomassie Brilliant Blue R-250 staining (A) and by Western blotting with a rabbit anti-ADA antibody (B). Lane 1: ADA only; lane 2: binding of serum DPP IV to ADA; lane 3: serum DPP IV only; lane 4: binding of kidney DPP IV to ADA; lane 5: kidney DPP IV only. Marker proteins used are catalase (232 kDa), lactate dehydrogenase (140 kDa), and bovine serum albumin (68 kDa).

and may be DPPT-L.

DPP IV Binding to Adenosine Deaminase—Human ADA exists in three forms: a low molecular weight type 1 (ADA-S, 40 kDa); a high molecular weight type 1 (ADA-L, 280 kDa); and an intermediate molecular weight type 2 (ADA-2, 110 kDa) (*42*). ADA-S predominates in almost all intracellular tissues and membranes, and is convertible to ADA-L, which is a complex of ADA-S (2 molecules) and DPP IV (1 molecule). On the other hand, ADA-2 is predominantly found in the serum and its properties have not yet been clarified. As shown in Fig. 5A, serum DPP IV (lane 3), like kidney DPP IV (lane 5), could exist as a dimer in non-SDS-PAGE gel. Upon incubation with ADA-S, the 220-kDa band of both serum and kidney DPP IVs disappeared and a new band with higher molecular weight appeared (lanes 2 and 4). Furthermore, in both serum and kidney DPP IVs, Western blotting using a rabbit IgG anti-ADA antibody showed the translocation of ADA from low molecular weight ADA (ADA-S) to the high molecular weight complex of DPP IV and ADA (ADA-L) (Fig. 5B). These results suggest that ADA-S can associate with both serum and membrane-bound DPP IV. Recently, some groups have reported that ADA-S interacts with the membrane form of DPP IV (*21, 43*), but Duke-Cohan *et al.* reported that DPP IV in human serum did not bind to ADA-S (*40*). From the point of view outlined above, we may question the sample which Duke-Cohan *et al.* used. Our result then suggests that DPP IV dose not need to be fixed on the plasma membrane in order to bind to ADA.

At present, we do not know the mechanism of the release from the membrane to serum, but it is worthwhile to clarify it and to investigate the correlation of serum DPP IV with immune status.

REFERENCES

1. Wu, Q., Lahti, J.M., Air, G.M., Burrows, P.D., and Cooper, M.D. (1990) Molecular cloning of the murine BP-1/6C3 antigen: A member of the zinc-dependent metallopeptidase family. *Proc. Natl. Acad. Sci. USA* **87**, 993-997
2. Nanus, D.M., Engelstein, D., Gastl, G.A., Gluck, L., Vidal, M.J., Morrison, M., Finstad, C.L., Bander, N.H., and Albino, A.P. (1993) Molecular cloning of the human kidney differentiation antigen gp160: Human aminopeptidase A. *Proc. Natl. Acad. Sci. USA* **90**, 7069-7073
3. Rogi, T., Tsujimoto, M., Nakazato, H., Mizutani, S., and Tomoda, Y. (1996) Human placental leucine aminopeptidase/oxytocinase: A new member of type II membrane-spanning zinc metallopeptidase family. *J. Biol. Chem.* **271**, 56-61
4. Laustsen, P.G., Rasmussen, T.E., Petersen, K., Pedraza-Diaz, S., Moestrup, S.K., Gliemann, J., Sottrup-Jensen, L., and Kristensen, T. (1997) The complete amino acid sequence of human placental oxytocinase. *Biochim. Biophys. Acta* **1352**, 1-7
5. Shipp, M.A., Richardson, N.E., Sayre, P.H., Brown, N.R., Masteller, E.L., Clayton, L.K., Ritz, J., and Reinherz, E.L. (1988) Molecular cloning of the common acute lymphoblastic leukemia antigen (CALLA) identifies a type II integral membrane protein. *Proc. Natl. Acad. Sci. USA* **85**, 4819-4823
6. Look, A.T., Ashmun, R.A., Shapiro, L.H., and Peiper, S.C. (1989) Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J. Clin. Invest.* **83**, 1299-1307
7. Ogata, S., Misumi, Y., and Ikehara, Y. (1989) Primary structure of rat liver dipeptidyl peptidase IV deduced from its cDNA and identification of the NH_2-terminal signal sequence as the membrane-anchoring domain. *J. Biol. Chem.* **264**, 3596-3601
8. Misumi, Y., Hayashi, Y., Arakawa, F., and Ikehara, Y. (1992) Molecular cloning and sequence analysis of human dipeptidyl peptidase-IV, a serine proteinase on the cell surface. *Biochim. Biophys. Acta* **1131**, 333-336
9. Tanaka, T., Camerini, D., Seed, B., Torimoto, Y., Dang, N.H., Kameoka, J., Dahlberg, H.N., Schlossman, S.F., and Morimoto, C. (1992) Cloning and functional expression of the T-cell activation antigen CD26. *J. Immunol.* **149**, 481-486
10. Shipp, M.A. and Look, A.T. (1993) Hematopoietic differentiation antigens that are membrane-associated enzymes: Cutting is the key. *Blood* **82**, 1052-1070
11. Hopsu-Havu, V.K. and Glenner, G.G. (1966) A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-β-naphthylamide. *Histochemie* **7**, 197-201
12. Walter, R., Simmons, W.H., and Yoshimoto, T. (1980) Proline specific endo- and exopeptidases. *Mol. Cell. Biochem.* **30**, 111-127
13. Tiruppathi, C., Miyamoto, Y., Ganapathy, V., Roesel, R.A., Whitford, G.M., and Leibach, F.H. (1990) Hydrolysis and transport of proline-containing peptides in renal brush-border membrane vesicles from dipeptidyl peptidase IV-positive and dipeptidyl peptidase IV-negative rat strains. *J. Biol. Chem.* **265**, 1476-1483
14. Barton, R.W., Prendergast, J., and Kennedy, C.A. (1990) Binding of the T cell activation monoclonal antibody Ta1 to dipeptidyl peptidase IV. *J. Leukocyte Biol.* **48**, 291-296
15. Torimoto, Y., Dang, N.H., Tanaka, T., Prado, C., Schlossman, S.F., and Morimoto, C. (1992) Biochemical characterization of CD26 (dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies. *Mol. Immunol.* **29**, 183-192
16. Schön, E., Jahn, S., Kiessig, S.T., Demuth, H.-U., Neubert, K., Barth, A., Von Baehr, R., and Ansorge, S. (1987) The role of dipeptidyl peptidase IV in human T lymphocyte activation. Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and immunoglobulin synthesis *in vitro*. *Eur. J. Immunol.* **17**, 1821-1826
17. Dang, N.H., Torimoto, Y., Deusch, K., Schlossman, S.F., and Morimoto, C. (1990) Comitogenic effect of solid-phase immobilized anti-1F7 on human CD4 T cell activation via CD3 and CD2 pathways. *J. Immunol.* **144**, 4092-4100
18. Bednarczyk, J.L., Carroll, S.M., Marin, C., and McIntyre, B.W. (1991) Triggering of the proteinase dipeptidyl peptidase IV (CD26) amplifies human T lymphocyte proliferation. *J. Cell. Biochem.* **46**, 206-218
19. Oravecz, T., Roderiquez, G., Koffi, J., Wang, J.H., Ditto, M.,

Bouhabib, D.C., Lusso, P., and Norcross, M.A. (1995) CD26 expression correlates with entry, replication and cytopathicity of monocytotropic HIV-1 strains in a T-cell line. *Nat. Med.* **1**, 919-926
20. Oravecz, T., Pall, M., Roderiquez, G., Gorrell, M.D., Ditto, M., Nguyen, N.Y., Boykins, R., Unsworth, E., and Norcross, M.A. (1997) Regulation of the receptor specificity and function of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) by dipeptidyl peptidase IV (CD26)-mediated cleavage. *J. Exp. Med.* **186**, 1865-1872
21. Morrison, M.E., Vijayasaradhi, S., Engelstein, D., Albino, A.P., and Houghton, A.N. (1993) A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. *J. Exp. Med.* **177**, 1135-1143
22. De Meester, I., Vanham, G., Kestens, L., Vanhoof, G., Bosmans, E., Gigase, P., and Scharpé, S. (1994) Binding of adenosine deaminase to the lymphocyte surface via CD26. *Eur. J. Immunol.* **24**, 566-570
23. Giblett, E.R., Anderson, J.E., Cohen, F., Pollara, B., and Meuwissen, H.J. (1972) Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* **2**, 1067-1069
24. Maes, M., Scharpe, S., De Meester, I., Goossens, P., Wauters, A., Neels, H., Verkerk, R., Demeyer, F., Dhondt, P., Peeters, D., Schotte, C., and Cosyns, P. (1994) Components of biological variation in prolyl endopeptidase and dipeptidylpeptidase IV activity in plasma of healthy subjects. *Clin. Chem.* **40**, 1686-1691
25. Ohkubo, I., Huang, K., Ochiai, Y., Takagaki, M., and Kani, K. (1994) Dipeptidyl peptidase IV from porcine seminal plasma: Purification, characterization, and N-terminal amino acid sequence. *J. Biochem.* **116**, 1182-1186
26. Kojima, J., Kanatani, M., Kato, M., Tojoh, F., and Nakamura, N. (1979) Serum glycylproline dipeptidyl aminopeptidase activity in human hepatic cancer. *Clin. Chim. Acta* **93**, 181-187
27. Hino, M., Nagatsu, T., Kakumu, S., Okuyama, S., Yoshii, Y., and Nagatsu, I. (1975) Glycylprolyl β-naphthylamidase activity in human serum. *Clin. Chim. Acta* **62**, 5-11
28. Fujita, K., Hirano, M., Ochiai, J., Funabashi, M., Nagatsu, I., Nagatsu, T., and Sakakibara, S. (1978) Serum glycylproline ρ-nitroanilidase activity in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Chim. Acta* **88**, 15-20
29. Iwaki-Egawa, S., Watanabe, Y., and Fujimoto, Y. (1995) Is CD26/dipeptidyl peptidase IV a really important molecule on T-cell activation in a certain strain rat? *Immunobiology* **194**, 429-442
30. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
31. Fahey, J.L. and Terry, E.W. (1973) in *Handbook of Experimental Immunology* (Weir, D.M., eds.) Vol. 1, Chapter 7, Blackwell Scientific Publications, Oxford
32. Watanabe, Y., Kojima, T., and Fujimoto, Y. (1987) Deficiency of membrane-bound dipeptidyl aminopeptidase IV in a certain rat strain. *Experientia* **43**, 400-401
33. Iwaki-Egawa, S., Watanabe, Y., and Fujimoto, Y. (1997) CD26/Dipeptidyl peptidase IV does not work as an adenosine deaminase-binding protein in rat cells. *Cell. Immunol.* **178**, 180-186
34. Iwaki-Egawa, S., Watanabe, Y., and Fujimoto, Y. (1993) N-Terminal amino acid sequence of the 60 kDa protein of rat kidney dipeptidyl peptidase IV. *Biol. Chem. Hoppe-Seyler* **374**, 973-975
35. Kato, T., Nagatsu, T., Fukasawa, K., Harada, M., Nagatsu, I., and Sakakibara, S. (1978) Successive cleavage of N-terminal Arg^1-Pro^2 and Lys^3-Pro^4 from substance P but no release of Arg^1-Pro^2 from bradykinin, by X-Pro dipeptidyl-aminopeptidase. *Biochim. Biophys. Acta* **525**, 417-422
36. Umezawa, H., Aoyagi, T., Ogawa, K., Naganawa, H., Hamada, M., and Takeuchi, T. (1984) Diprotins A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria. *J. Antibiot.* **37**, 422-425
37. Watanabe, Y., Iwaki-Egawa, S., Mizukoshi, H., and Fujimoto, Y. (1995) Identification of an alanine aminopeptidase in human maternal serum as a membrane-bound aminopeptidase N. *Biol. Chem. Hoppe-Seyler* **376**, 397-400
38. Watanabe, Y., Iwaki-Egawa, S., Yokosawa, J., Mizukoshi, H., and Fujimoto, Y. (1996) An antibody specific to N-terminal amino acid sequence of human maternal serum cystine aminopeptidase and its applications. *Biochem. Mol. Biol. Int.* **38**, 653-658
39. Watanabe, Y., Ito, K., Iwaki-Egawa, S., Yamaguchi, R., and Fujimoto, Y. (1998) Aminopeptidase N in sera of healthy subjects is a different N-terminal processed derivative from the one obtained from maternal serum. *Mol. Genet. Metab.* **63**, 289-294
40. Duke-Cohan, J.S., Morimoto, C., Rocker, J.A., and Schlossman, S.F. (1995) A novel form of dipeptidylpeptidase IV found in human serum: Isolation, characterization, and comparison with T lymphocyte membrane dipeptidylpeptidase IV (CD26). *J. Biol. Chem.* **270**, 14107-14114
41. Duke-Cohan, J.S., Morimoto, C., Rocker, J.A., and Schlossman, S.F. (1996) Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. *J. Immunol.* **156**, 1714-1721
42. Van der Weyden, M.B. and Kelley, W.N. (1976) Human adenosine deaminase distribution and properties. *J. Biol. Chem.* **251**, 5448-5456
43. Kameoka, J., Tanaka, T., Nojima, Y., Schlossman, S.F., and Morimoto, C. (1993) Direct association of adenosine deaminase with a T-cell activation antigen, CD26. *Science* **261**, 466-469