FEBS Letters 582 (2008) 819–825

Stromal cell-derived factors 1α and 1β, inflammatory protein-10 and interferon-inducible T cell chemo-attractant are novel substrates of dipeptidyl peptidase 8

Katerina Ajami[a,b], Melissa R. Pitman[c], Claire H. Wilson[c], Joohong Park[a,1], R. Ian Menz[c], Amanda E. Starr[d], Jennifer H. Cox[d], Catherine A. Abbott[c,2], Christopher M. Overall[d,2], Mark D. Gorrell[a,b,*,2]

[a] *Centenary Institute, Faculty of Medicine, University of Sydney, Locked Bag No. 6, Newtown, Sydney, NSW 2042, Australia*
[b] *A.W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Sydney, Australia*
[c] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[d] *University of British Columbia Centre for Blood Research, Department of Oral Biological and Medical Sciences and Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada*

Received 29 November 2007; revised 29 January 2008; accepted 1 February 2008

Available online 12 February 2008

Edited by Masayuki Miyasaka

Abstract **N-terminal truncation of chemokines by proteases including dipeptidyl peptidase (DP) IV significantly alters their biological activity; generally ablating cognate G-protein coupled receptor engagement and often generating potent receptor antagonists. DP8 is a recently recognised member of the prolyl oligopeptidase gene family that includes DPIV. Since DPIV is known to process chemokines we surveyed 27 chemokines for cleavage by DP8. We report DP8 cleavage of the N-terminal two residues of IP10 (CXCL10), ITAC (CXCL11) and SDF-1 (CXCL12). This has implications for DP8 substrate specificity. Chemokine cleavage and inactivation may occur in vivo upon cell lysis and release of DP8 or in the inactivation of internalized chemokine/receptor complexes.**

Keywords: Dipeptidyl peptidase 8; Chemokine; Substrate specificity; Dipeptidyl peptidase IV

1. Introduction

The dipeptidyl peptidase (DP) IV gene family contains four atypical serine proteases belonging to the prolyl oligopeptidase gene family [1]. This family is characterised by the catalytic triad serine-aspartic acid-histidine, with this order of residues being the reverse of that seen in typical serine proteases. The DPIV gene family has six members, four of which, DPIV, fibroblast activation protein, and the more recently discovered DP8 and DP9, possess DPIV enzyme activity. These enzymes have the rare enzyme ability to hydrolyse a prolyl bond two residues from the N-terminus of substrates [1–3].

Linking proteases with their substrates is crucial for understanding protease function [4]. DPIV is the best-studied member of its family. DPIV has roles in metabolism, immune responses, the endocrine system and cancer biology. Since glucagon-like peptide-1 (GLP-1) was in 1993 found to be a DPIV substrate much interest has been focused on the enzyme activity of DPIV, and its inhibition as a type 2 diabetes therapy [5]. Identification of DP8 and DP9 as enzymes possessing DPIV enzyme activity, previously assumed unique to DPIV, has stimulated investigations of DP8/9 functions and substrate specificity. This knowledge is crucial for further progress in selective targeting of DPIV family enzymes for disease therapies.

No in vivo functions of DP8 and DP9 have been delineated. A recent report has identified the known DPIV substrates GLP-1, GLP-2, neuropeptide Y (NPY) and peptide YY as in vitro substrates of DP8 and DP9 [6]. DP8 shows a kinetic favouring of substrates in the following order NPY > GLP-1 > GLP-2 ≫ PYY compared with DPIV which shows NPY ≈ PYY > GLP-1 > GLP-2. Although the substrate specificity of DP8 and DP9 is very similar to DPIV, their cleavage rates are slower than DPIV [6], so it is likely that the biologically preferred and relevant substrates of DP8 and DP9 are yet to be discovered. DP8 and DPIV both have a strong preference for proline in the P1 position [7]. However, DP8 has a strong preference for hydrophobic or basic residues at P2, in contrast to the much less discriminatory DPIV [7].

Chemokines regulate multiple cell functions, including cell chemotaxis, proliferation and apoptosis, as well as leukocyte trafficking regulation and homing to tissues. These biological activities are mediated through their interaction with G-protein coupled chemokine receptors expressed by target cells such as leucocytes and neural and endothelial cells [8]. Proteolytic truncation of chemokines has significant effects on their biological

*Corresponding author. Address: Centenary Institute, Faculty of Medicine, University of Sydney, Locked Bag No. 6, Newtown, Sydney, NSW 2042, Australia. Fax: +61 2 95656101.
E-mail address: m.gorrell@centenary.usyd.edu.au (M.D. Gorrell).

[1]Present address: Department of Medicine (Dermatology), University of Sydney, Australia.
[2]Equal senior authors.

Abbreviations: AFC, 7-amido-4-trifluoromethylcoumarin; CXCL, CXC ligand; CXCR, CXC chemokine receptor; GLP, glucagon-like peptide; HIV, human immunodeficiency virus; IP10, inflammatory protein-10; ITAC, interferon-inducible T cell chemo-attractant; DP, dipeptidyl peptidase; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight mass spectroscopy; pNA, *p*-nitroanilide; NPY, neuropeptide Y; PYY, peptide YY; SDF, stromal cell-derived factor

0014-5793/$34.00
doi:10.1016/j.febslet.2008.02.005

activity by modifying the binding capacity to their respective cognate chemokine receptors [1,9]. DPIV and matrix metalloproteinases (MMPs) have been shown to process the N-terminus of many chemokines thereby altering G-protein coupled receptor binding, activation and often desensitisation [9–14].

Biological functions of eight chemokines are known to be inactivated or altered following cleavage by DPIV. These include CXCL10 (inflammatory protein-10, IP10), CXCL11 (interferon-inducible T cell chemo-attractant, ITAC) and CXCL12 (stromal cell-derived factors 1α and 1β, SDF-1α and SDF-1β). For example, DPIV inactivates both SDF-1α and SDF-1β, by specifically cleaving their N-terminal dipeptide, which abrogates both their chemotactic and antiviral activities [14]. ITAC truncated by DPIV no longer induces extracellular signal-regulated kinase 1/2 or Akt/protein kinase B phosphorylation in CXC chemokine receptor-3 (CXCR3) transfected cells [13]. IP10 and ITAC signal via CXCR3. ITAC attracts activated T-helper type 1 lymphocytes and natural killer cells. ITAC truncation by DPIV results in loss of calcium binding though CXCR3 and more than 10-fold reduced chemotactic potency. Moreover, cleaved ITAC is a chemotaxis antagonist [13].

Certain chemokine receptors are coreceptors for human immunodeficiency virus type 1 (HIV-1) to enter susceptible CD4-expressing cells, so the cognate chemokines can competitively block virus entry [14]. CXCR4, the receptor for SDF-1, is a coreceptor for the T cell tropic strains of HIV-1 [15]. Interaction of CXCR4 with its ligand SDF-1 can block HIV-1 infection whereas SDF-1 proteolysis by DPIV or MMPs abrogates this protection [10,14]. SDF-1 also promotes tumour growth and malignancy, and is suggested to be involved in bone trophism of metastatic cells expressing CXCR4 [16]. Hence, inhibition of SDF-1 actions could be useful in developing novel anti-cancer therapies.

Here, we report testing 27 chemokines, 16 of which contain a penultimate proline, and demonstrate that, like DPIV, DP8 can remove the N-terminal dipeptides from SDF-1, IP10 and ITAC.

2. Materials and methods

2.1. Cloning, protein expression and purification

2.1.1. Expression of DP8 in Sf9 insect cells. DP8 (882aa) was cloned into the baculovirus pFastBacHTB vector using NcoI and XbaI sites. Constructs were transformed into MAX Efficiency® DH10Bac™ competent cells. Serum-free adapted Sf9 cells were grown in SF900™ III Media at 27 °C in suspension. Transfection with plasmid viral DNA used Cellfectin® and 2×10^6 cells/ml. Once cell viability reached 30% the supernatant was harvested by centrifugation and filtered (0.22 μM) then stored with 2% FCS. Virus amplification used the same method. Protein expression used a multiplicity of infection of 2 and harvesting cell pellets from 50 ml cells after 4 days. All reagents for DP8 expression were from Invitrogen (Carlsbad, CA).

2.1.2. Purification of DP8 protein from Sf9 insect cells. Protein pellets were suspended in Tris buffer (50 mM Tris–HCl, pH 8.0, 100 mM NaCl) containing 10 mM imidazole and the protease inhibitors 100 μM leupeptin (Sigma–Aldrich, St Louis, MO) and 20 μM E-64 (Sigma). Cells were lysed in a chilled French Press at 4000 psi. Following ultracentrifugation ($100000 \times g$, 30 min, 4 °C), lysate supernatant was loaded onto a 1 ml HisTrap nickel column (Amersham Biosciences, Little Chalfont, Bucks, UK) and washed with Tris buffer containing 10 mM then 30 mM imidazole. DP8 was eluted with 500 mM imidazole in Tris buffer and stored below 4 °C in 20% glycerol then later concentrated and buffer-exchanged using a 30 kDa spin column (Amicon, Houston, TX) into Tris buffer with 20% glycerol.

2.1.3. DP8 size exclusion chromatography. Size exclusion chromatography was carried out in Tris buffer on Sephadex 200 (Amersham) at a flow rate of 0.5 ml/min. The Sephadex column was calibrated with dextran blue (4000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa) and bovine serum albumin (66 kDa). Elution fractions were run on 7.5% SDS–PAGE under reducing conditions and transferred to PVDF membrane. Recombinant DP8 protein was detected using H-Ala-Pro-pNA and by immunoblot using an antibody to polyhistidine (Qiagen, Düsseldorf, Germany).

2.1.4. Expression and purification of DPIV. DPIV cloning, protein expression and purification has been described previously [17]. The DPIV construct, cDNA accession number M80536, lacked the cytoplasmic and transmembrane domains. This soluble construct encoded 744 amino acids in the pMelBacA vector that uses the honeybee mellitin secretion signal (Invitrogen). The DPIV was further purified using Superose 12 (Amersham) and 10 mM Tris, pH 8.0 then stored in 10% glycerol. A linear regression curve of protein standards was generated by plotting retention times against the log of the molecular mass of the calibration proteins immunoglobulin G (M_r 150000 Da), bovine serum albumin (M_r 67000 Da), cytochrome *c* (M_r 12600 Da) and cytidine (M_r 246 Da).

2.2. Kinetic constants

DP8 kinetics was tested in Tris buffer at 37 °C with eight concentrations of H-Ala-Pro-pNA (50 μl) (Bachem, Bubendorf, Switzerland). DP8 and DPIV proteins were diluted to 9.5 nM and 10 nM, respectively into a final assay volume of 100 μl. The data from three different protein batches and four independent experiments were analysed using non-linear regression of the Michaelis–Menten equation in the software Prism (v.4, GraphPad, San Diego, CA). DPIV was assayed with H-Gly-Pro-AFC and H-Ala-Pro-AFC at 20 °C in 10 mM Tris–HCl, 1 mM EDTA, pH 7.6.

2.3. Chemokine cleavage by DP8 and DPIV

The 27 chemokines were synthesized as described previously [18] and were identical to the intact counterparts found in humans (Table 1). The expected chemokine sizes were calculated using the in-house software PeptID. Chemokine (1.5 μg) was incubated with 0.082 μM DPIV, 1.11 μM DP8 or a quantity of the control vector preparation equivalent to the amount of DP8 for up to 24 h at 37 °C in 15 μl of 33 mM Tris–EDTA, pH 7.6. The calculated polypeptide masses were 88300 Da for DPIV and 104558 Da for DP8.

2.4. Matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF) analysis

MALDI-TOF was performed on a Voyager-DETM STR BioSpectrometry Workstation (Perseptive Biosystems Inc., Framingham, MA) equipped with a nitrogen laser (337 nm) running in linear mode with delayed extraction and ion acceleration at 25000 V. At each time point a sample of enzyme/chemokine solution was mixed with matrix solution in a 1:1 ratio then 1 μl of this mixture was applied to a standard stainless steel MALDI sample plate and allowed to air evaporate. Matrix solution consisted of 31–44 mM 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), 30% acetonitrile and 0.3% trifluoroacetic acid. Calibration was performed using calibration mixture 3 from the Sequazyme™ Peptide Mass Standards Kit (Applied Biosystems, Foster City, CA).

2.5. In vitro relative t1/2 life of DP8 truncated chemokines

The concentration of active DP8 was determined by active-site titration [10] to be 4.853 ± 1.066 μM. This assay used the slow-binding DPIV inhibitor isoleucyl-cyano-pyrrolidine (P59/99; Probiodrug, Halle, Germany) [19], which has a slow off-rate and acts as an irreversible inhibitor during a brief (<4 h) assay.

MALDI-TOF MS was used as described above to measure cleavage rates of chemokines by DP8. To estimate cleavage rates, chemokine at 12.032 ± 0.521 μM was incubated with purified active protein at 0.743 ± 0.136 μM (16:1 ratio of chemokine:DP8) in a total volume of 15 μl at 37 °C in 33 mM Tris–EDTA buffer, pH 7.6. Reaction samples of 1 μl were taken at 0, 0.25, 0.5, 2, 4, 8 and 24 h. Relative ratios and in vitro half-lives (*t*1/2) were estimated from ratios between the MS intensities of intact and cleaved chemokines after baseline correction and noise-filter/smoothing.

Table 1
Chemokine cleavage by DP8 and DPIV

Chemokine	Uniprot number	NH_2-terminal quadrapeptide	Number of amino acids	DP8 $\Delta[M+H]^+$	DPIV $\Delta[M+H]^+$
CXCL1/Groα	P09341	ASVA	73	nc	nc
CXCL2/Groβ	P19875	APLA	73	nc	143.6
CXCL3/Groγ	P19876	ASVV	73	nc	nc
CXCL5/ENA78	P42830	AGPA	78	nc	nc
CXCL6/GCP2	P80162	GPVS	77	nc	153.7
CXCL7/NAP2	P02775	AELR	70	nc	nc
CXCL8/IL-8	P10145	SAKE	72	nc	nc
CXCL9/MIG	Q07325	TPVV	104	nc	202.1
CXCL10/IP10	P02778	VPLS	77	203.0	197.8
CXCL11/ITAC	O14625	FPMF	73	245.7	246.1
CXCL12/SDF-1α	P48061	KPVS	67	226.8	228.2
CXCL12/SDF-1β	P48061	KPVS	72	228.5	226.6
CCL1/I309	P22362	KSMQ	73	nc	nc
CCL2/MCP1	P13500	QPDA	76	nc	nc
CCL3L1/MIP1α	P10147	APLA	73	nc	nc
CCL4/MIP1β	P13236	APMG	69	nc	nc
CCL5/ RANTES	P13501	SPYS	68	nc	nc
CCL7/MCP3	P80098	QPVG	76	nc	nc
CCL8/MCP2	P80075	QPDS	76	nc	nc
CCL11/Eotaxin	P61671	GPAS	74	nc	154.4
CCL13/MCP4	Q99616	QPDA	75	nc	nc
CCL14/HCC1	Q16627	TKTE	74	nc	nc
CCL15/HCC2	Q16663	QFIN	92	nc	nc
CCL16/HCC4	O15467	QPKV	97	nc	nc
CCL17/TARC	Q92583	ARGT	71	nc	nc
CCL23/MPIF	P55773	RVTK	99	nc	nc
CCL27/CTACK	Q9Y4X3	FLLP	88	nc	nc

N-terminal amino acid sequences where obtained using the Uniprot accession numbers listed at www.uniprot.org. Calculated mass is compared to the molecular mass observed by MALDI-TOF at commencement of and after incubation of each chemokine with each protein.
nc signifies that the peptide was not cleaved.

2.6. Silver staining

The samples were loaded onto 10% SDS–PAGE in 4× urea loading buffer. Pre-stained SeeBlue® Plus 2 (Invitrogen) was used as a molecular weight standard. Following electrophoresis, the gel was incubated in a Fixer solution (40% methanol, 10% acetic acid) for a minimum of 30 min. This was followed by 5 min incubation in a solution of 12 mM potassium hexacyanoferrate III (Sigma) and 25 mM sodium thiosulfate (Sigma). The gel was then rinsed three times with distilled water and incubated for a further 10 min in water. This process was repeated until the gel became clear. The gel was then incubated for 20 min in 12 mM silver nitrate solution. This was followed by two rinses in distilled water and two rinses in 2.9% sodium carbonate solution. The gel was transferred to a clean vessel and allowed to develop in developing solution. Once the desired intensity of the bands was reached, the incubation was stopped with 5% acetic acid solution.

3. Results

3.1. Recombinant soluble human DP8 and DPIV

DP8 was highly purified, active and dimeric. The purified recombinant human DP8 eluted from Sephadex 200 predominantly in its dimeric form, at approximately 220 kDa, but activity was observed in gel filtration fractions of greater size consistent with an active tetramer (Fig. 1A). On SDS–PAGE, the DP8 appeared as a monomer of approximately 110 kDa that accounted for 90% of the total protein (Fig. 1C and D). These biochemical observations on DP8 concord with previous indications that the native active form of DP8 is dimeric but the dimeric size is not evident on SDS–PAGE [7]. The K_m and k_{cat} of DP8 using H-Ala-Pro-pNA were 0.16 ± 0.07 mM and 10.3 ± 0.8 s^{-1}, with K_{cat}/K_m 65 s^{-1} mM^{-1}. Thus, this baculovirus – expressed purified recombinant human DP8 exhibited kinetic values consistent with those observed by others [6,7]. The purified recombinant soluble human DPIV showed a high level of purity and a size of approximately 180 kDa (Fig. 1B and C), which is the size of dimeric DPIV [20]. The K_m (mM) and k_{cat} (s^{-1}) for DPIV using H-Ala-Pro-AFC were 0.13 ± 0.042 and 25.9 ± 2.0, respectively (K_{cat}/K_m 199 ± 0.03 s^{-1} mM^{-1}), and 0.177 ± 0.28 and 30.9 ± 1.3, respectively on H-Gly-Pro-AFC [17].

3.2. Chemokine cleavage by DP8 and DPIV

The ability of DP8 and DPIV to hydrolyse naturally occurring peptides was tested three times using a MALDI-TOF MS based assay. Representative samples were taken at 0, 4, 8 and 24 h of peptide–enzyme co-incubation.

The four DP8-hydrolysed chemokines were SDF-1α, SDF-1β, IP10 and ITAC. MALDI-TOF data were obtained following incubations with each chemokine of up to 24 h with DP8 or DPIV. Significant cleavage of SDF-1α and SDF-1β was evident in the first minute of incubation with DPIV, whereas 1 h was the shortest incubation needed to detect truncated SDF-1 following incubation with DP8. Similarly, IP10 and ITAC were more rapidly hydrolysed by DPIV than DP8. Two preparations of DP8 produced similar data. Fig. 2 shows data derived from a single preparation of DP8.

Within 4 h of DPIV treatment eight chemokines exhibited size reductions consistent with removal of two N-terminal amino acids. These eight chemokines were SDF-1α, SDF-1β, IP10, ITAC, GCP2, Groβ, MIG and eotaxin. DPIV mediated hydrolysis of these eight chemokines has been reported previously [11,21,22]. No additional cleaved peptide sizes were detected during longer incubations with DPIV up to 24 h. There was no evidence of DPIV mediated cleavage in 19 of the 27 chemokines that were tested (Table 1). Unlike previous

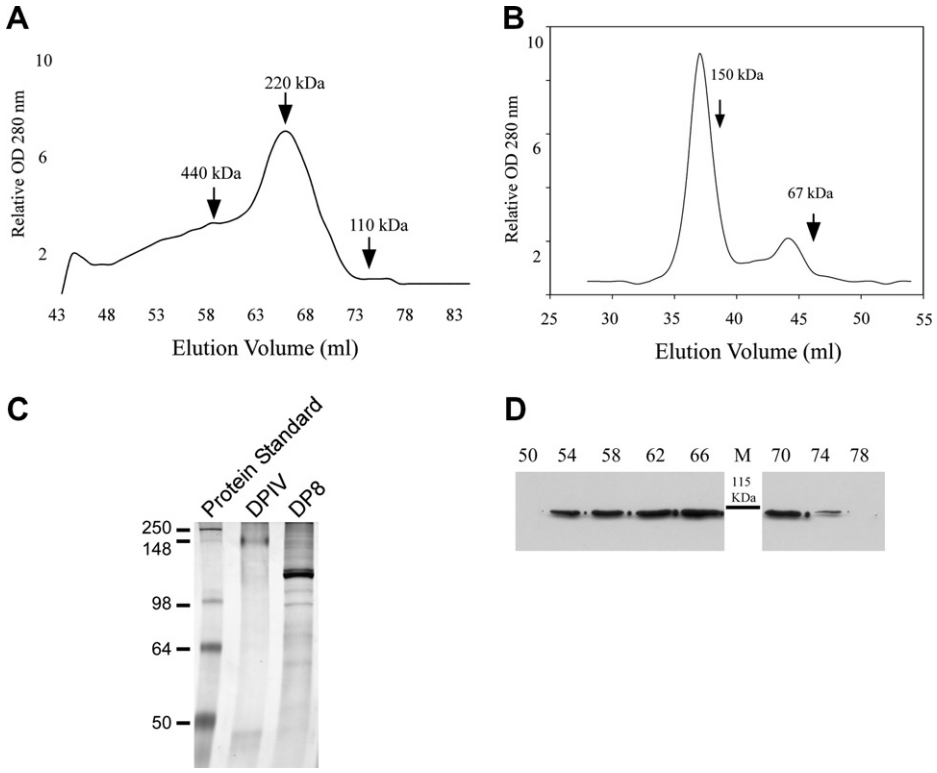

Fig. 1. Purity and size of DP8 and DPIV. (A) The elution profile of 1 mg of purified recombinant DP8 from size exclusion chromatography through Sephadex S200; arrows indicate DP8 locations and molecular mass deduced from calibration curve and immunoblot. DP activity was detected in fractions 52–74. (B) The elution profile of soluble recombinant DPIV from size exclusion chromatography through Superose 12; arrows indicate calculated molecular mass. (C) Purified DP8 and DPIV were the predominant bands on silver stained SDS–PAGE. (D) Immunoblot of DP8 gel filtration fractions 50–78 using an antibody to polyhistidine. The positions of molecular mass (kDa) marker proteins are shown.

reports [11,21], no cleavage of RANTES was observed in three replicate experiments.

Additionally, as a control procedure cell culture pellet derived from baculovirus-infected Sf9 cells was subjected to the purification procedure that was used to purify DP8. The product of this protocol, termed the vector control preparation, was incubated at a cell-equivalent dose with each chemokine for 24 h then subjected to MALDI-TOF analysis. This procedure showed that the dipeptidyl peptidase pattern of chemokine truncation by the DPIV and DP8 preparations was specific for those peptidases and not due to a co-purifying peptidase activity derived from infected Sf9 cells.

3.3. *In vitro kinetics of chemokine degradation by recombinant human DP8*

The relative *t*1/2 data obtained on the four chemokines shown to be hydrolysed by DP8 (Table 2) used a separate preparation of purified recombinant human DP8 in four replicate experiments. Negative controls included incubating chemokines alone and with the vector control preparation. Examples of MALDI-TOF data from these time course experiments form Supplemental figures 1 to 4.

4. Discussion

SDF-1α, SDF-1β, IP10 and ITAC are the first chemokine substrates of DP8 to be identified. These chemokines are known to be substrates of DPIV [11,21] and hydrolysis by DPIV was observed in this study. Unlike DPIV, DP8 did not hydrolyse GCP2, Groβ, and eotaxin. Moreover, DPIV hydrolysed chemokines more rapidly than did DP8. DPIV also exhibits greater catalytic efficiency than DP8 when removing the N-terminal dipeptide of NPY [23]. These data indicate that DPIV is more efficient than DP8 at catalyzing the cleavage of known DPIV substrates. The DP8 chemokine substrate degradome appears to be more restricted than that of DPIV and concords with the narrower P1 and P2 substrate specificity of DP8 compared to DPIV that has been obtained using artificial dipeptide substrates [6,7].

DP8 and DPIV are unusual enzymes in having the capacity to hydrolyse the prolyl bond. All the chemokines hydrolysed by DP8 or DPIV contain a penultimate proline. However, seven of the 16 chemokines that contained a penultimate proline were hydrolysed by neither DP8 nor DPIV. Therefore, proline in position P1 is insufficient to cause a natural peptide to be a DP8 or DPIV substrate. The DPIV catalytic pocket is small, about 8 Å across, and is inside a hollow protein that has a 15 Å – wide opening at the interface between its hydrolase and propeller domains [24]. The DP8 structure is predicted to be very similar [25,26]. These physical restrictions are thought to limit the substrate size but all chemokines are about 8 kDa, so examining 27 chemokines half of which have a proline in P1 provides interesting substrate specificity data. Using synthetic chromagenic dipeptides, a strong preference of DP8 for substrates with lysine or valine or phenylalanine at the P2 position has been demonstrated [7]. Similarly, the chemokines hydrolysed by DP8, which were SDF-1, IP10

and ITAC, contain lysine, valine or phenylalanine, respectively, at the P2 position. DPIV has high selectivity constants (k_{cat}/K_m) in the cleavage of SDF-1, IP10 and ITAC and a kinetic favouring in the order SDF-1 > ITAC > IP10 [21]. Our data suggest that DP8 differs a little in substrate preference, with a kinetic favouring of SDF-1 > IP10 ≫ ITAC. Our results suggest that the P1′ and P2′ residues might also have substrate specificity roles. ITAC, the least favoured substrate, has methionine in P1′ and phenylalanine in P2′, while SDF-1 and IP10 both contain serine in P2′ and leucine and valine in P1′, respectively (Table 1).

Interestingly, six of the twelve chemokines that have the preferred residue proline in the P1 position but are not cleaved by DP8 have serine, glycine or alanine at P2 even though these

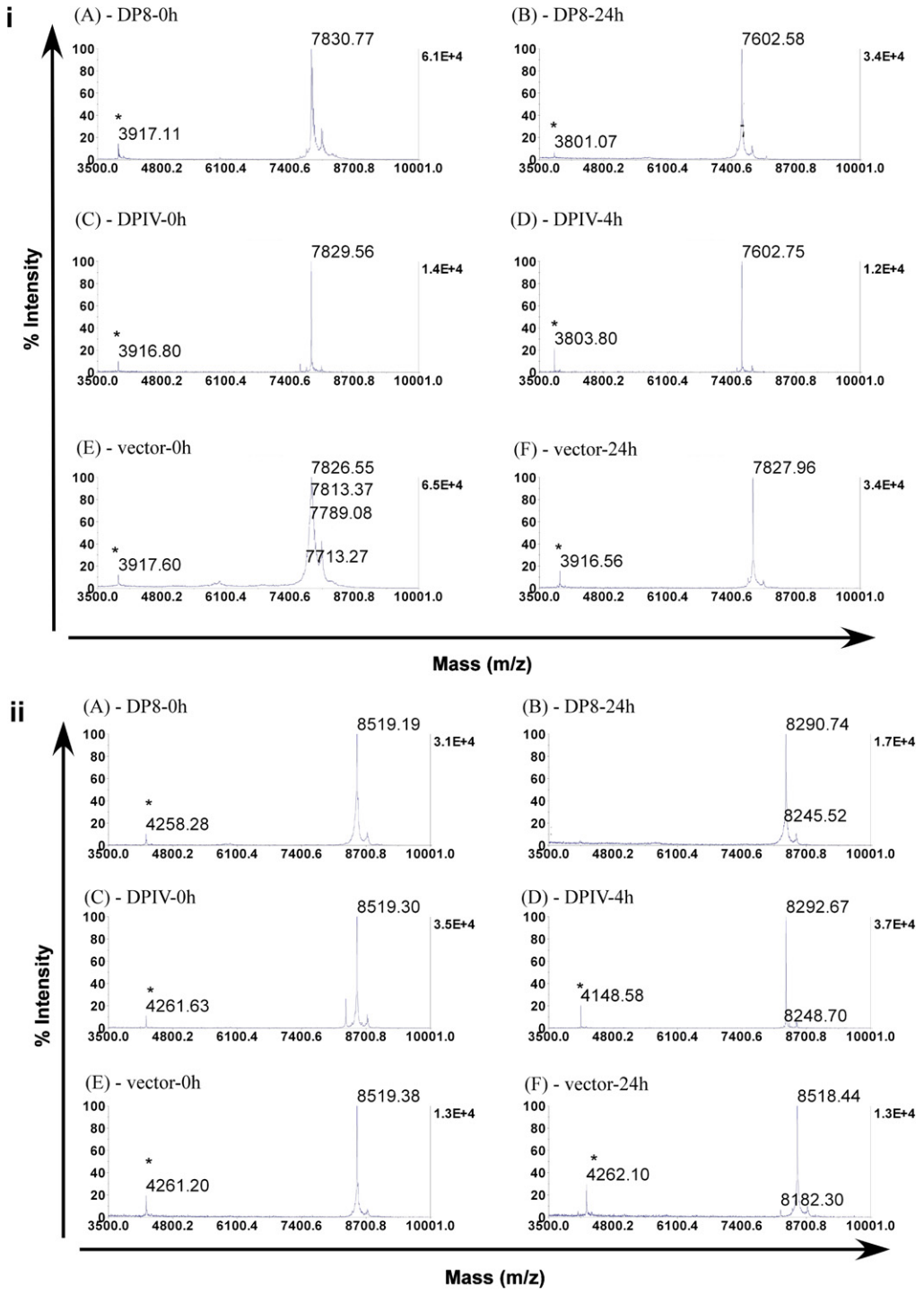

Fig. 2. Chemokine cleavage by DP8 and DPIV. Recombinant DP8 (A,B) and DPIV (C,D) were incubated with chemokine for 24 and 4 h, respectively. The vector control preparation (E,F) was incubated with chemokine for 24 h. DP8 cleaved SDF-1α (I), SDF-1β (II), IP10 (III), and ITAC (IV). Representative MALDI-TOF analyses of chemokine immediately before (A,C,E) and after (B,D,F) these incubations are shown ($n = 3$). Peaks are labeled with their molecular mass. Asterisks label double-charged chemokine peaks.

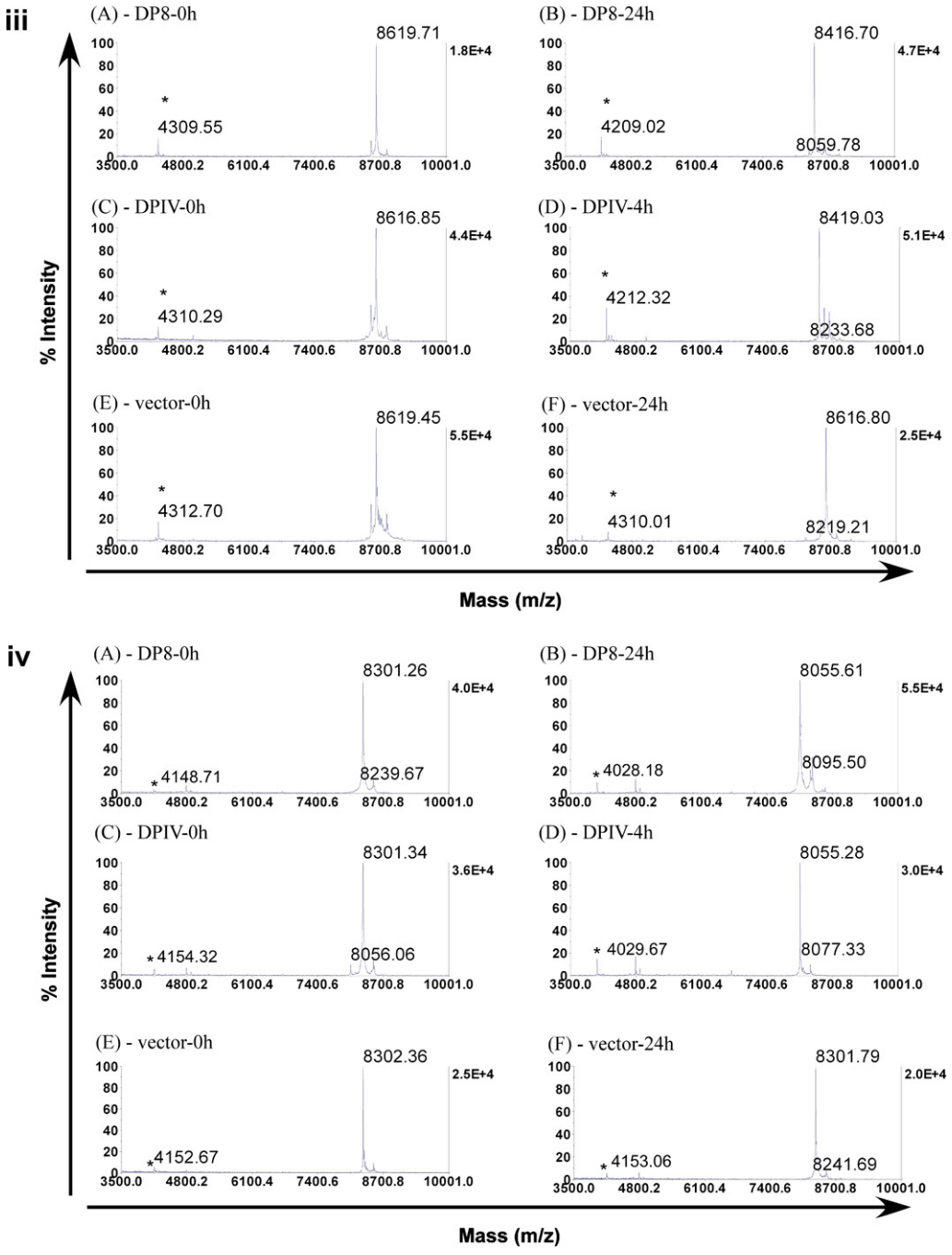

Fig. 2 (*continued*)

Table 2
Relative *t*1/2 life of DP8 truncated chemokines in vitro, using a 16:1 chemokine:DP8 ratio and MALDI-TOF analysis

Chemokine	Relative *t*1/2 (h)
CXCL12/SDF-1α (1–67)	4 ± 0.5
CXCL12/SDF-1β (1–72)	2 ± 0.5
CXCL10/IP10 (1–77)	13 ± 2.4
CXCL11/ITAC (1–73)	> 24

Data represent means ± S.D. ($n = 4$).

residues can be at P2 in synthetic chromagenic dipeptide substrates of DP8 [7]. This observation suggests that structural characteristics other than P1 and P2, perhaps including P1′ and P2′ as proposed above, influence DP8 substrate specificity. Further support for this hypothesis is provided by the observation that DP8 hydrolyses neuropeptide Y but not peptide YY. Neuropeptide Y and peptide YY are both hydrolysed by DPIV and have identical P1, P2 and P2′ residues but differ at P1′ (isoleucine in peptide YY and serine in neuropeptide Y) [6]. Exosite interactions have been shown to drive substrate hydrolysis or even negatively select chemokine substrates [27]. Thus, unfavourable exosite interactions may prevent cleavage of certain chemokines. Therefore, predicting cleavage based on the N-terminal sequence is difficult.

Whereas DPIV can access chemokines extracellularly, the intracellular location of DP8 makes it unclear whether DP8 makes physical contact with chemokines in vivo and so the biological relevance is unclear. Such uncertainty applies to most proteases where substrates have been identified only in in vitro assays [4]. Potentially upon cell death in inflammatory lesions DP8 released to the extracellular space would retain activity and process chemokines involved in these pathological lesions. Alternately, after triggering their cognate receptor, chemokines internalized with their receptors might be substrates for DP8.

DP8 is implicated in immune responses by its upregulation following T cell activation [2]. IP10 and ITAC have crucial roles in hepatitis C virus infection and DP8 is highly expressed in B-cell chronic lymphocytic leukaemia, various tumors and activated T cells, so this selective chemokine inactivation might have implications for cancer biology and immunobiology.

Acknowledgements: Supported by Australian National Health and Medical Research Council (NHMRC) Grant 293803 to M.D.G., NHMRC Program Grant 358398, a NHMRC postgraduate scholarship and a University of Sydney Faculty of Medicine Travel Award to K.A., and a Flinders University Overseas Field Trip Grant and a Masonic Foundation Trevor Prescott Memorial Scholarship to C.H.W. M.R.P. and C.H.W. hold Flinders University Research Scholarships. Support at UBC was from the Canadian Institutes of Health Research (CIHR) and a Centre Grant from the Michael Smith Research Foundation (C.M.O., J.H.C.), the Natural Sciences and Engineering Research Council of Canada (J.H.C., A.E.S.), CIHR Strategic Training Program STP-53877 (J.H.C., A.E.S.) and a Canada Research Chair in Metalloproteinase Proteomics and Systems Biology to C.M.O. Hans_Ulrich Demuth provided the inhibitor P59/99.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.02.005.

References

[1] Gorrell, M.D. (2005) Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin. Sci. 108, 277–292.
[2] Abbott, C.A., Yu, D.M.T., Woollatt, E., Sutherland, G.R., McCaughan, G.W. and Gorrell, M.D. (2000) Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. Eur. J. Biochem. 267, 6140–6150.
[3] Ajami, K., Abbott, C.A., McCaughan, G.W. and Gorrell, M.D. (2004) Dipeptidyl peptidase 9 has two forms, a broad tissue distribution, cytoplasmic localization and DPIV-like peptidase activity. Biochim. Biophys. Acta 1679, 18–28.
[4] Overall, C.M. and Blobel, C.P. (2007) In search of partners: linking extracellular proteases to substrates. Nat. Rev. Mol. Cell. Biol. 8, 245–257.
[5] Thornberry, N.A. and Weber, A.E. (2007) Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Curr. Top. Med. Chem. 7, 557–568.
[6] Bjelke, J.R., Christensen, J., Nielsen, P.F., Branner, S., Kanstrup, A.B., Wagtmann, N. and Rasmussen, H.B. (2006) Dipeptidyl peptidase 8 and 9 specificity and molecular characterization compared to dipeptidyl peptidase IV. Biochem. J. 396, 391–399.
[7] Lee, H.J., Chen, Y.S., Chou, C.Y., Chien, C.H., Lin, C.H., Chang, G.G. and Chen, X. (2006) Investigation of the dimer interface and substrate specificity of prolyl dipeptidase DPP8. J. Biol. Chem. 281, 38653–38662.
[8] Rossi, D. and Zlotnik, A. (2000) The biology of chemokines and their receptors. Annu. Rev. Immunol. 18, 217–242.
[9] McQuibban, G.A., Gong, J.-H., Tam, E., McCulloch, C.A.G., Clark-Lewis, I. and Overall, C.M. (2000) Inflammation dampened by gelatinase a cleavage of monocyte chemoattractant protein-3. Science 289, 1202–1206.
[10] McQuibban, G.A., Butler, G.S., Gong, J.-H., Bendall, L., Power, C., Clark-Lewis, I. and Overall, C.M. (2001) Matrix metalloproteinase activity inactivates the chemokine stromal-cell derived factor-1. J. Biol. Chem. 276, 43503–43508.
[11] Oravecz, T. et al. (1997) Regulation of the receptor specificity and function of the chemokine RANTES (regulated on activation normal T cell expressed and activated) by dipeptidyl peptidase IV (CD26)-mediated cleavage. J. Exp. Med. 186, 1865–1872.
[12] Proost, P., Menten, P., Struyf, S., Schutyser, E., De Meester, I. and Van Damme, J. (2000) Cleavage by CD26/dipeptidyl peptidase IV converts the chemokine LD78 beta into a most efficient monocyte attractant and CCR1 agonist. Blood 96, 1674–1680.
[13] Proost, P. et al. (2007) Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signalling and reduces lymphocyte and endothelial cell migration. Blood 110, 37–44.
[14] Shioda, T. et al. (1998) Anti-HIV-1 and chemotactic activities of human stromal cell-derived factor 1alpha (SDF-1alpha) and SDF-1beta are abolished by CD26/dipeptidyl peptidase IV-mediated cleavage. Proc. Natl. Acad. Sci. USA 95, 6331–6336.
[15] Oberlin, E. et al. (1996) The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 382, 833–835.
[16] Kryczek, I., Wei, S., Keller, E., Liu, R. and Zou, W. (2007) Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am. J. Physiol. Cell Physiol. 292, C987–C995.
[17] Park, J., Ajami, K. and Gorrell, M.D. (2006) Dipeptidyl peptidase 8 has post-proline dipeptidyl aminopeptidase and prolyl endopeptidase activities in: Dipeptidyl Aminopeptidases in Health and Disease II (Lendeckel, U., Reinhold, D. and Bank, U., Eds.), pp. 93–102, Kluwer, New York.
[18] Clark-Lewis, I., Vo, L., Owen, P. and Anderson, J. (1997) Chemical synthesis, purification, and folding of C–X–C and C–C chemokines. Methods Enzymol. 287, 233–250.
[19] Hoffmann, T. and Demuth, H.U. (2002) Therapeutic strategies exploiting DP IV inhibition – target disease: type 2 diabetes in: Ectopeptidases: CD13/Aminopeptidase N and CD26/Dipeptidylpeptidase IV in Medicine and Biology (Langner, J. and Ansorge, S., Eds.), pp. 259–278, Kluwer, New York.
[20] Ajami, K., Abbott, C.A., Obradovic, M., Gysbers, V., Kähne, T., McCaughan, G.W. and Gorrell, M.D. (2003) Structural requirements for catalysis, expression and dimerisation in the CD26/DPIV gene family. Biochemistry 42, 694–701.
[21] Lambeir, A.M. et al. (2001) Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. J. Biol. Chem. 276, 29839–29845.
[22] Proost, P. et al. (2001) Amino-terminal truncation of CXCR3 agonists impairs receptor signaling and lymphocyte chemotaxis, while preserving antiangiogenic properties. Blood 98, 3554–3561.
[23] Frerker, N. et al. (2007) Neuropeptide Y (NPY) cleaving enzymes: structural and functional homologues of dipeptidyl peptidase 4. Peptides 28, 257–268.
[24] Rasmussen, H.B., Branner, S., Wiberg, F.C. and Wagtmann, N. (2003) Crystal structure of human DPP-IV/CD26 in complex with a substrate analogue. Nat. Struct. Biol. 10, 19–25.
[25] Pitman, M.R., Menz, R.I. and Abbott, C.A. (2006) Prediction of dipeptidyl peptidase 8 structure by homology modelling in: Dipeptidyl Aminopeptidases: Basic Science and Clinical Applications (Lendeckel, U., Reinhold, D. and Bank, U., Eds.), pp. 33–42, Kluwer, New York.
[26] Rummey, C. and Metz, G. (2007) Homology models of dipeptidyl peptidases 8 and 9 with a focus on loop predictions near the active site. Proteins 66, 160–171.
[27] Overall, C.M., McQuibban, G.A. and Clark-Lewis, I. (2002) Discovery of chemokine substrates for matrix metalloproteinases by exosite scanning: a new tool for degradomics. Biol. Chem. 383, 1059–1066.