Kinetic Investigation of Chemokine Truncation by CD26/Dipeptidyl Peptidase IV Reveals a Striking Selectivity within the Chemokine Family*

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Chemokines coordinate many aspects of leukocyte migration. As chemoattractants they play an important role in the innate and acquired immune response. There is good experimental evidence that N-terminal truncation by secreted or cell surface proteases is a way of modulating chemokine action. The localization of CD26/dipeptidyl peptidase IV on cell surfaces and in biological fluids, its primary specificity, and the type of naturally occurring truncated chemokines are consistent with such a function.

We determined the steady-state catalytic parameters for a relevant selection of chemokines (CCL3b, CCL5, CCL11, CCL22, CXCL9, CXCL10, CXCL11, and CXCL12) previously reported to alter their chemotactic behavior due to CD26/dipeptidyl peptidase IV-catalyzed truncation. The results reveal a striking selectivity for stromal cell-derived factor-1α (CXCL12) and macrophage-derived chemokine (CCL22). The kinetic parameters support the hypothesis that CD26/dipeptidyl peptidase IV contributes to the degradation of certain chemokines in vivo. The data not only provide insight into the selectivity of the enzyme for specific chemokines, but they also contribute to the general understanding of CD26/dipeptidyl peptidase IV secondary substrate specificity.

Chemokines coordinate leukocyte migration required for development, differentiation, tissue localization, immune surveillance, and effector function. As chemoattractants, they play an essential role in the innate and acquired immune response (1–4). This is achieved through activation of seven transmembrane domain G protein-coupled receptors expressed on the target cells. Several chemokines bind to multiple receptors, and the receptors often respond to more than one chemokine (5, 6). Chemokine receptors are exploited by HIV1 for cell entry and disease transmission. Many chemokines provide a certain degree of protection against HIV infection (7–10).

The amino-terminal part of the chemokines is involved in key interactions with their receptors. For a number of chemokines, modification or proteolytic removal of the first few N-terminal amino acids leads to significant changes in receptor selectivity and functional activity (1). Protease-induced changes in receptor selectivity have consequences for the type of cells that will be attracted and/or activated by the chemokines. Chemokines were isolated as both intact and truncated forms from natural sources (11–17). Thus, secreted and cell surface chemokines, by their ability to remove N-terminal amino acids, contribute to the regulation of cell traffic. CD26/dipeptidyl peptidase IV (CD26/DPP IV) fulfills all the requirements for such a regulatory function (18, 19). This membrane bound extracellular peptidase cleaves dipeptides from the N terminus of polypeptide chains, preferably after a proline or alanine but under certain conditions other amino acids may be accepted (20). Originally described as a T-cell activation molecule, CD26 is now regarded as a nonlineage antigen, whose expression is regulated by the differentiation and activation status of immune cells. Although CD26 is absent on resting B and NK cells, it is induced on their surface upon stimulation. In resting peripheral blood mononuclear cells, a small subpopulation of T-cells expresses CD26 at high density on the surface (CD26 bright T cells) (21). This subset displays a number of defined phenotypic (co-expression with CCR5 and LFA-1) and functional characteristics (19, 21–23). CD26 bright T-cells proliferate strongly in response to soluble antigens and alloageneic cells, secrete Th1 type cytokines, and have transendothelial migration capacity (24–26). Several distinct anti-CD26 mAbs have costimulatory activities in anti-CD3-driven activation of purified T-cells (22). There are conflicting in vitro data on the role of the enzymatic activity of CD26 in immune regulation (27, 28). In general, there are good arguments to believe that chemokines encounter CD26/DPP IV during their life span, since the protein is not only expressed on activated T-lymphocytes but also on endothelial cells, fibroblasts, and epithelial cells.

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The amino acid sequence of this protein can be accessed through NCBI Protein Database under NCBI accession numbers P27487 (DPP IV/CD26), A28845 (RANTES), O00626 (MDC), P16619 (LD78), P48061 (SDF-1α), P51671 (etoxacin), O14625 (I-TAC), P02778 (IP-10), and Q67225 (Mig).

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¶ The abbreviations used are: HIV, human immunodeficiency virus; DPP IV, dipeptidyl peptidase IV (EC 3.4.14.5); I-TAC (CXCL11), interferon-inducible T cell α-chemoattractant; IP-10 (CXCL10), γ-interferon-inducible protein 10; MCP-2 (CCL8), monocyte chemotactic protein-2; MDC (CCL22), macrophage-derived chemokine; MDC69, intact MDC molecule containing 69 amino acids; MDC67, MDC molecule missing the two N-terminal amino acids and containing 67 amino acids; Mig (CXCL9), monokine induced by γ-interferon; RANTES (CCL5), regulated upon activation, normal T-lymphocyte expressed and presumably secreted; SDF-1α (CXCL12), stromal cell-derived factor-1α; HPLC, high pressure liquid chromatography.
cells, which all play a role in cell migration. Moreover, CD26/DPP IV is present in a catalytically active soluble form in plasma (29).

In a continuing attempt to unravel the in vivo functions of CD26/DPP IV, several research groups studied the hydrolysis of natural substrates. The pancreatic polypeptide family including neuropeptide Y and peptide YY are well characterized substrates, as are several members of the glucagon family (e.g. glucagon, glucagon-like peptide-1, and glucagon-like peptide-2). For most of the above, kinetic parameters were determined (30).

That CD26/DPP IV is able to cleave certain chemokines is well substantiated (12, 31–42). It is important to realize that it is not a common feature of all chemokines, since only a fraction of them contain the N-terminal X-Pro or X-Ala motif preferred by CD26/DPP IV. Even then, some proved resistant to truncation (36). Previously, we and others investigated the in vitro ability of CD26/DPP IV to truncate chemokines as well as the effect of truncation on the chemokine activities. In some cases, complete removal of the N-terminal dipeptide required a large amount of enzyme and inordinately long incubation times. This raises the questions of whether chemokines may serve as substrates in vivo and whether CD26/DPP IV has a preference for some chemokines over others.

The aim of this investigation is to determine the catalytic parameters for a relevant selection of chemokines previously reported to alter their chemotactic behavior due to CD26/DPP IV-catalyzed truncation (for reviews, see Refs. 19 and 30).

The data not only provide insight in the selectivity of the enzyme for specific chemokines, but they also contribute to the understanding of CD26/DPP IV substrate specificity in general.

**EXPERIMENTAL PROCEDURES**

**Materials**—RANTES, MDC69, MDC67, etoxacin, IP-10, I-TAC, Mig, and SDF-1α were obtained from Peprotec Inc. (Rock-Hill, NJ). LD78β was synthesized as described (42). Neuropeptide Y was purchased from Sigma. Gly-Pro-p-nitroanilide, Ala-4-methoxy-2-naphthylamide, and commercially available chromogenic substrates were from Sigma or Bachem (Bubendorf, Switzerland). Other p-nitroanilide substrates (X-Pro-p-nitroanilide) were synthesized using established procedures. The N-terminal nonapeptides of various chemokines were synthesized using an automated Finnssolid phase peptide synthesizer (Rainin Instrument Company Inc., Woburn, MA). The irreversible CD26/DPP IV inhibitor bis(4-acetamidoephosphonate) 1-(S)-(S)-pyrrolidino-2(R,S)-phosphate hydrochloride was synthesized as described (43).

**Protein Purification and Activity Measurements**—Soluble CD26/DPP IV was purified from human seminal plasma as described before (44, 45). The specific activity of the enzyme was 35 units/mg. Contaminating aminopeptidase activity (measured with 1 mM Ala-4-methoxy-2-naphthylamide) was less than 0.1%. Enzymatic activity was determined kinetically at 37 °C using the chromogenic substrate Gly-Pro-p-nitroanilide (0.5 mM) in 40 mM Tris/HCl buffer, pH 8.3, by monitoring the release of p-nitroaniline at 405 nm (SpectraMax340 microplate reader; Molecular Devices, Inc., Sunnyvale, CA). One unit of activity is defined as the amount of enzyme that cleaves 1 μmol of substrate/min under these conditions. Protein concentrations were determined using BCA reagent with bovine serum albumin as a standard. The theoretical molecular mass of soluble CD26/DPP IV is 85,123 Da per subunit.

**Inhibition Measurements**—Inhibition constants of the nonapeptides were determined in the presence of 0.5 mM Gly-Pro-p-nitroanilide (S). Assuming competitive inhibition, the affinity constant of the peptides for the active site (Kᵢ) was calculated from the IC₅₀ value as follows,

\[ K_i = \frac{IC_{50}}{1 + [S]} \]  

(Eq. 1)

where \( K_i \) is the concentration of nonapeptide required to obtain 50% reduction of the activity in standard assay conditions.

**Kinetics of Nonapeptide Degradation**—The cleavage of nonapeptides (5 mM) by CD26/DPP IV (typically 265 units/liter) was monitored as a function of time in 100 mM Tris/HCl buffer, pH 7.5, 1 mM EDTA at 37 °C. EDTA was added to eliminate contaminating aminopeptidase activity. At regular time intervals, samples (40 μl) were taken, quenched in 0.1% trifluoroacetic acid, and analyzed by HPLC as described before (40).

**Kinetics of Chemokine Truncation**—To establish the optimal conditions for a kinetic study, RANTES, MDC69, MDC67, etoxacin, IP-10, I-TAC, Mig, SDF-1α, and LD78β were incubated at a concentration of 5 μM with increasing concentrations of CD26/DPP IV (0.25, 2.5, 25, and 250 units/liter) in 50 mM Tris/HCl buffer, pH 7.5, 1 mM EDTA at 37 °C. At certain time intervals, samples (5 μl) were withdrawn and quenched in 0.1% trifluoroacetic acid. Incubation with buffer alone and equal amounts of CD26/DPP IV previously inactivated by the addition of the bis(4-acetamidoephosphonate) 1-(S)-(S)-pyrrolidino-2(R,S)-phosphate inhibitor served as negative controls to verify the specificity of the reaction. The samples were desalted using C18 ZipTips (Millipore Corp., Bedford, MA) and eluted with 50% acetonitrile, water, 0.1% acetic acid. The composition of the mixture was determined with an Esquire LC Ion Trap mass spectrometer (Bruker, Bremen, Germany). The instrument was used in a wide range, normal resolution setting, optimized on the m/z value of the most abundant ion of the intact chemokine. For the deconvolution, at least three peaks were used. The abundance cut-off was set at 5%.

To determine the steady-state kinetic parameters, different concentrations of chemokine (0.5–20 μM) were incubated with the appropriate amount of CD26/DPP IV to obtain about 25% conversion between 5 and 30 min. The ratio of the abundance of the intact and the cleaved forms was used to calculate the amount of substrate converted. Since the method was not well suited for the quantification of very small percentages of conversion, the average rate of conversion \((\frac{|S|_i - |S|_f}{t_f - t_i})\) was plotted versus the average substrate \((\frac{|S|_{ave}}{t_f - t_i})\).

\[ \frac{|S|_i - |S|_f}{t_f - t_i} = \frac{V_{max} |S|_{ave}}{K_m + |S|_{ave}} \]  

(Eq. 2)

where \(|S|_{ave} = (|S|_i + |S|_f)/2\). The results were analyzed by direct fitting to the Michaelis-Menten equation.

**RESULTS**

**Specificity of Cleavage and Interpretation of Mass Spectra**—The specificity of the reaction between CD26/DPP IV and various chemokines was confirmed by determining the composition of the reaction mixture in function of time. The observed molecular mass of intact and truncated forms is shown in Table I. In all cases, cleavage occurred after the penultimate proline, except for MDC, where a second cleavage occurred after the glycine on position 4. Truncation was prevented by preincubation of the enzyme with an irreversible CD26/DPP IV inhibitor. Other types of proteolytic activity were not observed, even after prolonged incubation with a very high CD26/DPP IV concentration (e.g. 30 min with 250 units/liter). Also shown in Table I is the composition of the commercial chemokine preparations. In general, the major molecule had the expected molecular mass, indicating that the recombinant chemokines were properly processed, i.e. without N-terminal methionine. I-TAC was 19 Da smaller than expected from its amino acid sequence. The MDC preparations contained a significant amount of a molecule that was too small by 28 Da. Since both species were efficiently degraded by CD26/DPP IV, the mass spectra only provided a relative abundance of the molecules that were present. In order to further identify the amount of chemokine converted at various time points during the reaction with CD26/DPP IV, it was necessary to establish that the signal was proportional to the concentration and that this proportionality factor was the same for the intact and truncated molecules. Equal volumes of chemokine dilutions, that had been incubated with either an excess of CD26/DPP IV or the incubation buffer, were mixed and
Kinetics of CD26/DPP IV-catalyzed Chemokine Truncation

**TABLE I**

| Chemokine | Sequence | Calculated $M_r$ | Measured $M_r$ | Ions in spectrum | Truncated/Intact $^a$ |
|-----------|----------|-----------------|----------------|-----------------|---------------------|
| SDF-1α    | KPVSLSYRC- | 7959.4          | 7961.1 ± 0.4   | +7 to +12       | 0.9 |
|           | VPVSLSYRC- | 7758.1          | 7735.7 ± 0.3   |                 |         |
| MDC69     | GPYGNMED- | 8065.0          | 8087.8 ± 1.4 (78) | +6 to +11       | 1.3 |
|           | YGNMED-   | 7932.3          | (7932.5 ± 1.2 (90)) |               | 1.3 |
|          | ANMED-    | 7712.1          | 7712.2 ± 0.9 (80) |               | 1.0 |
| MDC67     | YGNMEDSV- | 7932.3          | 7932.5 ± 1.2 (100) | +6 to +11       | 1.6 |
|           | ANMEDSV-  | 7712.1          | 7704.3 ± 1.0 (30) |               | 1.0 |
| I-TAC     | FPMFKGRGRC- | 8307.0         | 8288.7 ± 0.4 (100) | +7 to +14       | 1.1 |
|           | MFKGRGRC- | 8062.8          | 8044.8 ± 0.6 (100) |               | 1.0 |
| IP-10     | VPLSRTVRC- | 8617.3          | 8618.1 ± 0.3   | +8 to +14       | 1.0 |
|           | LSRTVRC-  | 8421.1          | 8421.1 ± 0.5   |               | 1.0 |
| Mig       | TPVVRKGRC- | 11720.8         | 11722.5 ± 1.5 (100) | +12 to +19      | 1.0 |
|          | VVRKGRC-  | 11522.6         | 11524.5 ± 0.8 (100) |               | 1.0 |

$^a$ Theoretical average relative molecular mass of molecules in the chemokine preparation.

$^b$ Measured average relative molecular mass. The relative abundance is given between brackets.

$^c$ The observed relative abundance of the truncated form and the intact form in a 50/50 mixture (with and without CD26/DPP IV incubation).

$^d$ Transiently observed molecules shown in brackets.

desalted, and the ratio of the truncated over the intact form was determined. The experimental ratio approached 1 (Table I), with the exception of MDC, where an overestimation of the truncated form is possible.

**Kinetics of Chemokine Conversion**—The mass spectra obtained at various time points during the conversion of SDF-1α are shown in Fig. 1. The time course of truncation was determined with the chemokine at 5 μM and a CD26/DPP IV concentration in the normal range for human plasma (25 units/liter). The degradation curves of SDF-1α, MDC, eotaxin, and RANTES are shown in Fig. 2. Catalytic rate constant ($k_{cat}$) and $K_m$ were determined from the concentration dependence of the conversion rate, such as shown in Fig. 3. The kinetic parameters and the half-life of the chemokines are listed in Table II. For comparison, we also studied (by HPLC) the conversion of nonapeptides corresponding to the nine N-terminal amino acids of the chemokines of this study, as well as a series of X-Pro-p-nitroanilide substrates, with X representing the amino-terminal residue of the chemokines. The truncation rate of the nonapeptides at 5 mM concentration was used to calculate the catalytic rate constant ($k_{cat}$), and the inhibition constant ($K_i$) determined in the presence of a chromogenic substrate was used to estimate the affinity for the active site ($K_m$).

Fig. 4 allows the comparison of the chemokine parameters with reported values of several other substrates at a glance. Chemokines and nonapeptides in the top panel and other bioactive peptide substrates in the lower panel are identified by *symbols and characters* as explained in the figure legend. Every symbol has a unique combination of $k_{cat}$ and $K_m$ values that can be read (on a logarithmic scale) on the $y$ and $x$ axis, respectively. Substrates with the same $k_{cat}/K_m$ ratio will be positioned on a diagonal line, such as the solid line representing $k_{cat}/K_m = 10^6$ M$^{-1}$ s$^{-1}$. A recurring problem with the interpretation of catalytic rate constants is that it requires the absolute enzyme concentration, and differences may arise from variations in specific activity and molecular weight estimates. To allow direct comparison of our data with known substrates, the catalytic parameters of neuropeptide Y were determined with the same enzyme preparation as the chemokines in identical conditions of pH, buffer composition, and temperature. They are depicted by the asterisk (*) in both panels of Fig. 4. The theoretical average relative molecular mass of molecules in the chemokine preparation.

**DISCUSSION**

As with all bioactive peptides that exert their function at very low concentrations (low nM for chemokines), the most relevant kinetic parameter is the selectivity constant ($k_{cat}/K_m$). This parameter can be considered as the second order rate constant of substrate binding to the free enzyme and determines the efficiency of the enzymatic conversion at subsaturating concentrations. The upper limit of $k_{cat}/K_m$ is the diffusion-controlled association of the substrate (around $10^{10}$ M$^{-1}$ s$^{-1}$ for large molecules), but values in the range 10$^5$ to 10$^6$ M$^{-1}$ s$^{-1}$ are more common. Most chemokines of this study have values in a range considered physiologically significant (30). At a given enzyme concentration with [S] $< K_m$, the half-life of the substrate is only determined by the $k_{off}/K_m$ value and is independent of the substrate concentration. The data in Table II predict a half-life of less than 1 min for SDF-1α in plasma. MDC, I-TAC, and IP-10 are degraded in less than 15 min, while Mig...
and eotaxin may take several hours. The low value of $k_{\text{cat}}/K_m$ observed for LD78/H9252 and RANTES is rather surprising. As a consequence, in vivo truncation of SDF-1/H9251, MDC, I-TAC, IP-10, Mig, and eotaxin by local CD26/DPP IV is realistic. This hypothesis is strengthened by the natural occurrence of N-terminally truncated forms for most of the chemokines chosen for this study (specifically SDF-1/H9251, MDC, I-TAC, eotaxin, RANTES, and LD78/H9252) (11–13, 15, 16, 46–48). In the test tube, MDC is degraded in consecutive steps. Because there is almost a factor of 10 difference in $k_{\text{cat}}/K_m$ between the two reactions, a significant amount of MDC-(3–69) accumulates (Fig. 2). This is consistent with the recovery of MDC-(3–69) and MDC-(5–69) in preparations from biological sources (11, 12).

In order to study whether the variation in primary structure in the neighborhood of the scissile bond could explain the differences in kinetic parameters, we studied the hydrolysis of nonapeptides whose sequences correspond to the chemokine N termini. Fig. 4 allows the comparison of catalytic parameters of chemokines and their N-terminal nonapeptides. As a group, the chemokines have lower $K_m$ and $k_{\text{cat}}$ values than the peptides. For a number of chemokines (e.g. eotaxin and IP-10), the $k_{\text{cat}}/K_m$ values are in the same order for the peptides. This indicates that the N-terminal part of these chemokines is rather flexible (as expected for a short peptide in solution) and easily accessible for the enzyme. The $K_m$ values of the chemokines vary with a factor 25 between the lowest and the highest value. The $K_m$ values of the nonapeptides are at least 10-fold larger than the $K_m$ values for the intact chemokines and show a similar range of variation. The $K_m$ values of chromogenic substrates (X-Pro-p-nitroanilide), only differing by the nature of the N-terminal amino acid, are in the same range as those of the nonapeptides and show a 5-fold variation (Table II). The
Kinetics of CD26/DPP IV-catalyzed Chemokine Truncation

Comparison between the kinetic parameters of chemokines and nonapeptides (upper panel) and reported natural CD26/DPP IV substrates (lower panel). Chemokines are separated from their N-terminal nonapeptides by lower \( k_{\text{cat}} \) and \( K_m \) values. The chemokines cluster with the best natural substrates reported for CD26/DPP IV. The \( k_{\text{cat}} \) values are displayed on the y axis versus the \( K_m \) values on the x axis on a logarithmic scale. The \( k_{\text{cat}}/K_m \) values between \( 10^6 \) and \( 10^7 \) \( M^{-1} \cdot s^{-1} \) are located between the two dotted parallel diagonal lines. The solid line represents \( k_{\text{cat}}/K_m = 10^6 \) \( M^{-1} \cdot s^{-1} \). The top panel shows the data for the chemokines (○) and the derived N-terminal nonapeptides (●). A, α, SDF-1α; B, b, MDC69; C, MDC67; D, d, I-TAC; E, e, IP-10; F, f, Mig; G, g, eotaxin; H, h, RANTES; I, LD785. The asterisk represents the neuropeptide Y parameters determined in the conditions of this study. The lower panel shows the reported values for relevant CD26/DPP IV substrates as follows: neuropeptide Y (○), peptide YY (+), substance P (●), substance P (3–11) (△), growth hormone-releasing factor (⊙), glucose-dependent insulinotropic peptide (☐), glucagon-like peptide 1 (□), peptide histidine methionine (★), and glucagon (◆) (Refs. 20, 30, 54, and 55 and references therein).
constant of around 200 μM (49, 50). In both SDF-1α and eotaxin, the N terminus is flexible and freely accessible even in the dimer structure. The low $k_{\text{cat}}/K_m$ value of eotaxin on the one hand and the high selectivity constant of SDF-1α on the other hand reflect the intrinsic range of CD26/DPP IV cleavage efficiency for chemokine monomers. Under the conditions of our study, RANTES and LD78β form stable aggregates (51). Their N-terminal amino acids are engaged in interactions in the dimer interface (51, 52). Therefore, it must be assumed that only the few free monomers in solution react with CD26/DPP IV. Thus, aggregation of the chemokines is expected to artificially lower the apparent values of $k_{\text{cat}}$ and $K_m$ by decreasing the effective substrate concentration. The parameters listed in Table II for LD78β and RANTES are therefore lower estimates for the values of the monomeric forms. Our data are compatible with a model where the dimerization is readily reversible, since RANTES and LD78β could be completely converted to their truncated forms and the observed conversion rate was dependent on the CD26/DPP IV concentration. We found that the N-terminally truncated RANTES-(3–68) acts as a more potent suppressor of HIV infection than intact RANTES. Most interestingly, intact RANTES did not suppress HIV infection in CD26 negative cell lines, but this could be corrected by the addition of external CD26/DPP IV (32). This finding together with the isolation of RANTES-(3–68) from natural sources, suggests truncation of RANTES by CD26/DPP IV in the presence of physiological levels of DPP IV activity. LD78β, a human homologue of murine MIP-1α, has an even lower $k_{\text{cat}}/K_m$ value. It differs from MIP-1α notably by a substitution of Ser-2 by a proline, which in theory makes it a DPP IV preferred substrate (53).

Chemokines with a natural N-terminal modification escape CD26/DPP IV. When recombinant MCP-2 is produced, the N-terminal glutamine spontaneously forms a pyroglutamate, but the conversion is not always complete. Incubation with CD26/DPP IV caused the unmodified molecule to disappear, while the pyroglutamate variant remained intact. Therefore, it was concluded that the biologically active MCP-2 (with a pyroglutamate) is not a substrate for CD26/DPP IV (36). The results of our kinetic study suggest that presentation of a free and accessible N terminus is an important determinant for selectivity.

The kinetic parameters of the chemokines can also be compared with those of other physiological substrates for CD26/DPP IV, such as the neuropeptides (neuropeptide Y and peptide YY) and the glucagon-like family of peptides (Fig. 4). In general, large (>20 amino acids) bioactive peptides have lower $k_{\text{cat}}$ and $K_m$ values than shorter presumably unstructured peptides. X-Ala chromogenic substrates and tripeptides are cleaved with significantly less efficiency than the X-Pro analogues. Surprisingly, the X-Pro chemokines studied here and other X-Pro substrates have similar catalytic parameters as the incretins and glucagon, which are cleaved after alanine and proline, which in theory makes it a DPP IV preferred substrate (56).

I-TAC > IP-10 > Mig > eotaxin > RANTES > LD78β follows more or less the truncation of the nonapeptides, except for RANTES, but not of the X-Pro-p-nitroanilide (Pro > Ala > Val > Tyr > Ser > Lys > Phe > Gly > His > Ile > Asn > Asp). The P3 side chains that occur in the natural substrates obey the specificity described for tripeptides and dipeptide derivatives (i.e. large hydrophobic and aromatic side chains, lysine, and small amino acids) (18, 30). With Pro in P1, we find on P1′ primarily large hydrophobic/aromatic side chains and alanine. The reported inhibition of CD26/DPP IV by tripeptides and longer peptides containing the X-X-Pro motif indicates that the substrate binding pocket can accommodate a proline in the P1′ position, but the X-Pro bond is not hydrolyzed (57, 58). In contrast, the known natural substrates with alanine on P1′ (all closely related) have Asp, Glu, or Gln on P1′. The P1′ position is often occupied by small amino acids (Gly, Ser, Ala). On P3′, there are a variety of amino acids with small polar, long aliphatic and positively charged side chains. P3′ is characterized by polar, positively and negatively charged side chains. In P3′, no clear pattern could be recognized except for a prevalence of Thr at P3′. The crystal structure of a related enzyme, prolyl oligopeptidase, with a nonapeptide bound to the active site, was recently published (59). In this complex, only two amino acids were discernible following the scissile bond (P1′ and P2′). The P3′ residues were disordered and could not be placed in the electron density map. Our results indicate that the substrate recognition by CD26/DPP IV does not extend much further than P3′. The recognition pattern described above is presumably biased, because the N-terminal amino acids also reflect the functional requirements of the chemokines and the other natural substrates (i.e. receptor binding and triggering).

In conclusion, we have demonstrated that several individual chemokines are good substrates for CD26/DPP IV. The selectivity of the enzyme is determined, on the one hand, by the availability of a freely accessible N terminus and, on the other hand, by elements of the chemokine’s structure. The chemokines that are converted with the highest efficiency are SDF-1α, MDC, and I-TAC. Coincidentally, these three chemokines have a restricted receptor specificity (CXCR4 for SDF-1α, CCR4 for MDC, and CXCR3 for I-TAC). They are the most attractive targets to assess the effect of CD26/DPP IV truncation in vivo.

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