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Distinctive pH Dependence and Substrate Specificity of Peptide Hydrolysis by Human Stromelysin-1

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Abstract

A kinetic profile of the catalytic domain of stromelysin-1 (SCD) using the fluorescent peptide substrate has been determined by the stopped-flow technique. The pH profile has a pH optimum of about 5.5 with an extended shoulder above pH 7. Three pK_a values, 5.0, 5.7, and 9.8 are found for the free enzyme state and two pH independent k_{cat}/K_m values of 4.1×10^4 M^{-1} s^{-1} and 1.4×10^4 M^{-1} s^{-1} at low and high pH, respectively. The profile is quite different in shape with other MMP family which has been reported, having broad pH optimum with two pK_a values. The substrate specificity of SCD towards fluorescent heptapeptide substrates has been also examined by thin layer chromatography. The cleavage sites of the substrates have been identified using reverse-phase HPLC method. SCD cleaves Dns-PLA↓L↓WAR and Dns-PLA↓L↓FAR at two positions. However, the Dns-PLA↓LRAR, Dns-PLE↓LFAR, and Dns-PLS_{ar}↓LFAR are cleaved exclusively at one bond. The double cleavages of Dns-PLALWAR and Dns-PLALFAR by SCD are in marked contrast to the close structurally related matrilysin. A notable feature of SCD catalysis agrees with the structural data that the S_1' pocket of SCD is deeper than that of matrilysin. The differences observed between SCD and matrilysin may form the basis of understanding the structural relationships and substrate specificities of the MMP family *in vivo*.

Key words – matrix metalloproteinase; zinc metalloenzyme; fluorescent peptide substrate, pH dependence

Introduction

Human stromelysin-1 (MMP-3) is a member of the group of matrix metalloproteinases (MMPs). This specific member has a broad substrate specificity towards the components of the extracellular matrix (collagens, gelatins, laminin, fibronectin, proteoglycans)[1,21] and is also reported to be involved in the activation process of inactive pro-MMPs[13,14,23]. Like the other members of the MMPs, stromelysin-1 is involved in a number of normal processes, including morphogenesis, bone and uterine absorption and wound healing. Besides, an elevated MMP concentration has been measured in connection with diseases such as rheumatoid arthritis and cancer, which strongly implies that these enzymes play a crucial role in the fatal development of these diseases[8,18,19]. In recent years this recognition has initiated a large research effort in the field of drug design in order to develop suitable drugs for MMP inhibition. An important part of this process is the study of the mechanism of hydrolysis on a molecular basis in order to learn the details in the catalysis and enzyme-substrate interactions. A proposed mechanism for substrate hydrolysis in MMPs is the

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"General base" mechanism which has previously been suggested for related hydrolytic zinc enzymes such as carboxypeptidase A[7,17] and thermolysin [22,29]. This assumption is mainly based on topological similarities of the active centers between these enzymes. Addition to these structural comparison, a basic understanding of the kinetic properties of the MMP family is also essential to elucidate their physiological actions as well as drug discovery.

We have initiated studies on a truncated stromelysin-1 (SCD) which contains solely the catalytic domain in its active form in order to learn about its structure to function relationships [6]. Native full-length MMPs are reported to be susceptible to autocatalytic degradation due to the presence of several putative cleavage sites in the C-terminal domain[20]. The reported crystal structure of full-length collagenase from pig has revealed that the C-terminal domain is linked to the N-terminal (catalytic) domain via a solvent exposed Proline-rich peptide which explains the susceptibility for autocatalytic cleavage in the linking peptide region[16]. In contrast, MMPs containing solely the catalytic domain are reported to be less susceptible to autolysis and can be stored for a prolonged length of time at 4°C[32]. SCD was synthesized as an active recombinant enzyme which opens up the possibilities to use the same construct to make mutant forms[32]. No self activation is needed when the enzyme is expressed in the active form. In the present study we have investigated the kinetic pH profile of SCD using a fluorescent heptapeptide as a substrate and compared with matrilysin. Our study focuses on the distinctive feature of SCD in the substrate specificity and pH dependence with relevance to the importance of the enzyme-substrate interactions of MMPs *in vivo*.

Materials and Methods

Materials

Expression and purification of SCD was carried out as previously described[32]. Enzyme concentrations were determined by amino acid analysis (AccQTaq methodology). Fluorescent peptides were prepared using Merrifield's solid-phase peptide synthesis method as described [2,26]. Substrate concentrations were determined spectrophotometrically by measuring the absorbance of the danyl group at 340 nm where ε_{340} = 4,300 M^{-1} cm^{-1} [28]. All solutions were made up in Milli-Q water from a Millipore reagent system. All buffers were of analytical grade, purchased from Sigma.

Stopped-Flow Experiments

A kinetic profile of the SCD catalyzed hydrolysis of Dns-PLALWAR was obtained on a Durrum D110 stopped-flow instrument interfaced to a PDP 11/34 DEC computer. Trp is excited at 290 nm and fluoresce at 340 nm. The dansyl group absorbs at around 340 nm and hence quenches the Trp fluorescence. The reaction could thus be monitored by measuring the marked increase in fluorescence at 340 nm upon cleavage between A−L or L−W bond using a 340 nm band pass filter. Kinetic parameters were obtained by measuring initial rates corresponding to less than 5% substrate conversion after a 1:1 mixing of substrate and enzyme solutions. All assays were repeated 4−5 times and averaged. The substrate and enzyme concentrations were ~5.0×10^{-6} M, and ~9.3×10^{-7} M, respectively. Acetate, Mes, Hepes, and Ampso were used in the pH ranges 4.5−5.0, 5.5−6.5, 7−8.5, and 9−10, respectively. Final buffer conditions were 50 mM buffer, 0.5 M NaCl, 10 mM $CaCl_2$, and 2.5% acetonitrile. $ZnCl_2$ was added to a concentration of 5×10^{-5} M in the pH region 4.5−6 in order to compensate thermodynamically for the increased competition at low pH between hydrogen ions and zinc ion. All samples were thermostated to 25±0.2°C prior to data collection. The k_{cat}/K_m values were obtained directly from the Michaelis-Menten equation: $v_{init} = k_{cat} [E][S] / ([S]+K_m)$ which is equal to $k_{cat}/K_m [E][S]$ when $[S] << K_m$.

TLC Assays

The possibility of a double-site cleavage of the substrates Dns-PLALWAR, Dns-PLALFAR, Dns-PLALRAR, Dns-PLELFAR, and Dns-PLS$_{ar}$LFAR was checked by a thin layer chromatography (TLC) microassay (Micropolyamide plates, Schleicher & Schuell)[2,26]. TLC assays were conducted at room temperature. The reaction was initiated by adding 2 μl of enzyme stock solution to 18 μl substrate solution. The final concentrations of enzyme and substrate were 1×10^{-7} M and 1×10^{-4} M, respectively in 50 mM Hepes, 0.15 M NaCl, 10 mM $CaCl_2$, 5% (v/v) acetonitrile at pH 7.5. Approximately 0.5−1 μl aliquots were spotted onto a TLC plate. The substrate and product migrate according to the degree of hydrophobicity when the plate is placed in an aqueous solution of 0.3 N HCl. Typically, the substrate (e.g. Dns-PLALWAR) remained at the bottom line whereas the more hydrophilic hydrolyzed products (e.g. Dns-PLA or Dns-PLAL) migrated with the solvent border line. The sheet was subsequently dried by heat and the dansylated substrate and product were visualized under a long-wave UV lamp.

HPLC Assays

The Waters HPLC system used for assays was composed of two model 510 pumps, a U6K injector, an extended wavelength module, a model 440 absorbance detector, an automated gradient controller, a model 745 datamodule integrator, a model 710B wisp automatic sample injection system, and a Novapac analytical C-18 column (3.9×15 mm). The determination of the cleavage sites for SCD-hydrolyzed dansyl substrates were performed at 25±0.2°C. Typically, a 300 μl assay contained 1×10^{-4} M Dns-PLALWAR substrate in the presence of 50 mM Hepes, 0.15 M NaCl, 10 mM $CaCl_2$, 5% (v/v) acetonitrile, pH 7.5. The enzyme concentration was 1×10^{-7} M. Samples of 40 μl were withdrawn after 1 hour incubation and the reaction was quenched in 20 μl glacial acetic acid. The substrate and product were eluted using a linear acetonitrile gradient of 20−60% buffer B in 25 min. Buffer A was Milli-Q water containing 0.1% (v/v) TFA and buffer B was 80% (v/v) acetonitrile in Milli-Q water containing 0.085% (v/v) TFA. Buffers were degassed on the same day of use. The elution was monitored at 214 nm.

Results

pH Dependence of SCD

The pH dependence of SCD hydrolysis of Dns-PLALWAR was measured at a substrate concentration of at least a factor of 10 below K_m in order to obtain k_{cat}/K_m value directly from initial rate measurements. Examination of the rate dependence as a function of NaCl concentration reveal no salt effect in the range 0−0.5 M NaCl (data not shown). A concentration of 0.5 M was chosen for pH dependence studies to ensure constant ionic strength when varying pH. It was checked that 10 mM $CaCl_2$ was sufficient to maintain maximum activity at low and high pH. Addition of $ZnCl_2$ at low pH rests on a similar pH dependence study of the matrilysin catalyzed hydrolysis of Dns-PLALWAR[5]. Addition of acetonitrile to 2.5% (v/v) ensure total solubility of the substrate. A circular dichroism spectrum for SCD (240−340 nm) in the absence and presence of acetonitrile to a concentration of 5% (v/v) reveals no change in the tertiary structure upon addition of acetonitrile. Fig. 1 shows the measured k_{cat}/K_m values plotted against pH. The profile has a distinguished feature with an acidic pH optimum of about 5.5. An extended shoulder ranging from pH 7 to around 8.5 defines a plateau and the curve declines again above pH 9. The data are fitted to equation 1.

$$k_{cat}/K_{m(obs)} = (k_{cat}/K_m)_1 / ([H^+]/K_{a1} + 1 + K_{a2}/[H^+] + K_{a2}K_{a3}/[H^+]^2) + (k_{cat}/K_m)_2 / ([H^+]^2/K_{a1}K_{a2} + [H^+]/K_{a2} + 1 + K_{a3}/[H^+]). \quad (1)$$

The equation reflects that four ionization states are involved (Scheme 1). $k_{cat}/K_{m(obs)}$ is the measured k_{cat}/K_m

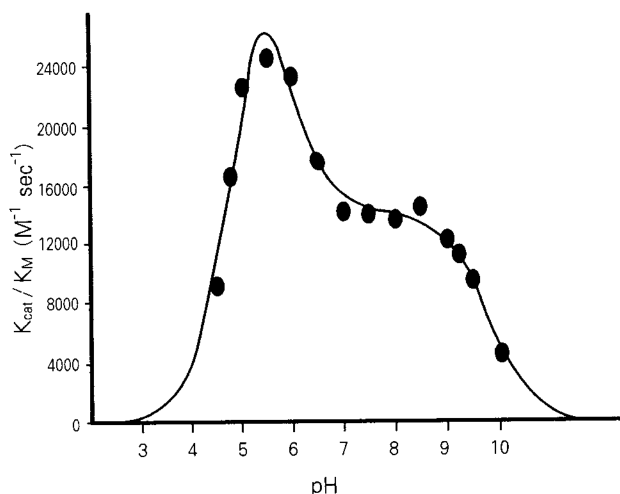
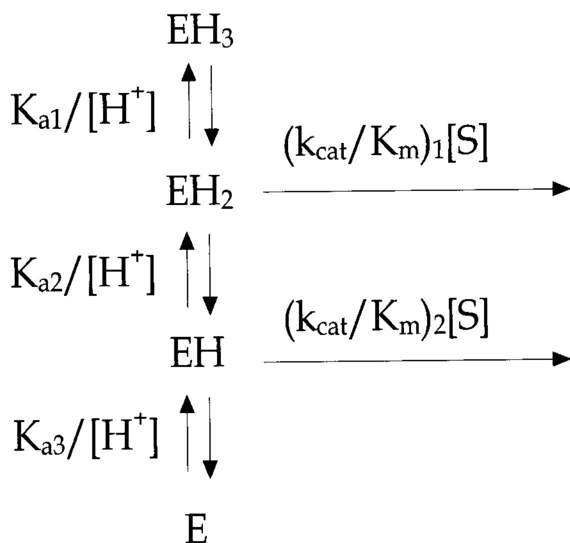


Fig. 1. pH Dependence of k_{cat}/K_m for SCD using the peptide substrate Dns-PLALWAR.
The solid line represents computer fit to the model shown in Scheme 1.



Scheme 1

value at different pH values, and the $(k_{cat}/K_m)_1$ and $(k_{cat}/K_m)_2$ are two pH independent parameters. K_{a1}, K_{a2}, and K_{a3} are the acid dissociation constants for free enzyme at low, medium, and high pH, respectively.

The resulting three pK_a values for the free SCD are thus 5.0, 5.7, and 9.8 and two pH-independent k_{cat}/K_m values, 4.1×10^4 $M^{-1}sec^{-1}$ and 1.4×10^4 $M^{-1}sec^{-1}$ (Table 1).

Table 1. pH Dependence of k_{cat}/K_m for the SCD-catalyzed hydrolysis of peptide

parameter	SCD	matrilysin
pK_{a1}	5.0 ± 0.1	4.3 ± 0.1
pK_{a2}	5.7 ± 0.2	NA
pK_{a3}	9.8 ± 0.1	9.6 ± 0.1
$(k_{cat}/K_m)_1$	4.1×10^4 $M^{-1}s^{-1}$	NA
$(k_{cat}/K_m)_2$	1.4×10^4 $M^{-1}s^{-1}$	NA
(k_{cat}/K_m)	NA[a]	9.8×10^4 $M^{-1}s^{-1}$

[a]Not available

Cleavage Specificity of SCD

A TLC microassay has confirmed that SCD can cleave the dansylated peptides at specific position as seen by the separate product spots on the TLC plate. The cleavage sites of the substrate were identified by HPLC using a linear acetonitrile gradient of 20−60% buffer B in 25 minutes. All eluted product fractions were concentrated by lyophilization and analyzed by amino acid analysis. The determined cleavage sites by SCD are shown in Table 2. The results reveal that the substrate Dns-PLALWAR and Dns-PLALFAR are cleaved at both A−L and L−W/F bond whereas the other substrates having either a Glu or Sar at the P_1 site or an Arg at the P_2' site are cleaved exclusively after Ala/Glu/Sar. The peptide substrates Dns-PLALWAR and Dns-PLALFAR are cleaved at the unusual position between Leu and Trp/Phe. This cleavage pattern is unique compared to the matrilysin which cleaves only one site between Ala

Table 2. Peptide substrate specificity of SCD and matrilysin[a]

	P_3	P_2	P_1		P_1'		P_2'	P_3'	P_4'
Dns -	P	L	A	↓	L	⇓	W	A	R
Dns -	P	L	A	↓	L	⇓	F	A	R
Dns -	P	L	A	↓	L	R	A	R	
Dns -	P	L	E	↓	L	F	A	R	
Dns -	P	L	Sar	↓	L	F	A	R	

[a]cleavage site was identified by HPLC method
↓; Cleavage site by SCD and matrilysin
⇓; Cleavage site by SCD only

and Leu in both substrates. The different substrate specificity against two substrates might be caused by the different structure of the S_1' pocket of stromelysin-1 and matrilysin.

Discussion

The truncated stromelysin-1 lacking the C-terminal domain (SCD) was used in this study because the deletion of C-terminal domain has been shown to have little effect on the ability of stromelysin-1 to cleave synthetic peptides and macromolecular substrates[20,31, 32]. SCD was expressed and purified to greater than 95% homogeneity, as judged by SDS-PAGE. A kinetic pH profile of the SCD catalyzed hydrolysis of Dns-PLALWAR has been obtained in the pH region 4.5−10. The profile has a distinguished feature not observed for other MMPs such as matrilysin[5,9], porcine synovial collagenase-1, and gelatinase[27] but similar in shape to a previously reported profile for a truncated stromelysin-1[11]. It reflects more complex reaction pattern, compared to a single bell-shaped profile, of which two ionization states of the enzyme (EH and EH_2) can catalyze the reaction as depicted in Scheme 1. A pH optimum close to 5.5 is observed here and an extended shoulder ranging from pH 7 to 8.5 defines a plateau and the curve declines again above pH 9. The pK_a values found in the present study are 5.0, 5.7, and 9.8[11]. It is quite different with the pH profile of matrilysin which has pK_a values of 4.3 and 9.6 (Table 1).

The identity of groups responsible for pK_{a1} and pK_{a3} in the MMP family remains unsettled. We previously assigned pK_{a1} in matrilysin to Glu-198[5]. This residue is analogous to Glu-143 in thermolysin, which had been assigned to similar acidic pK_a value in this enzyme[15]. However, the mutagenesis study of Glu-198 in matrilysin reevaluated the acidic pK_a of MMP family as an ionization of zinc-bound water[4]. Thus, it now seems likely that pK_{a1} in the MMP family is due to the zinc-bound water. The alkaline pK_a value is still undecided since the ionization of putative Tyr-223 previously assigned as an alkaline pK_a is unaffected by mutation of this residue [3]. The complex pH profile of SCD is unique among the MMPs in that ionization of pK_{a2} is required to explain its pH profile. The pK_{a2} value of 5.7 is ascribed to deprotonation of His-224 because the predicted pK_a of imidazole of histidine is consistent with the observed pK_{a2} value and His-224 is unconserved residue located in a relatively flexible loop that forms part of its S_1' site which is crucial for enzyme-substrate interactions[10]. This prediction was recently confirmed by the mutation study of Tyr-224 of truncated stromelysin-1[12]. The mutation by glutamine residue eliminated the shoulder of the wild-type enzyme and showed bell-shaped pH profile similar to the other MMPs. The pH profile shown in Fig. 1. indicates the average k_{cat}/K_m value of double cleavage at A−L and L−W bond. In the first case Leu occupies the deep S_1' pocket, whereas Trp occupies the pocket when the substrate is cleaved between Leu and Trp. The total profile can be split into two parts after HPLC product analysis. The two profiles have the same overall feature as seen for the "full" pH profile (data not shown).

The hydrolysis of dansyl substrates by SCD reveals an interesting characteristics in substrate specificity. TLC microassay shows that the enzyme cleaves Dns-PLALWAR at the A-L bond and L-W bond simultaneously. The same cleavage pattern is found for the substrate Dns-PLALFAR (F instead of W). However, the only one cleavage site between A and L is found for the hydrolysis of the other substrates, Dns-PLALRAR, Dns-PLELFAR, and Dns-PLS_{ar}LFAR. Previously, substrate specificity studies of human MMPs have suggested that a Pro is favored in the S_3 position[24,25]. Thus, in this case the S_1' pocket is occupied by a Leu and the cleavage is between Ala and Leu. However, the fact that the substrate can also be cleaved between L and W suggests that the pocket has a high affinity towards bulky aromatic hydrophobic side chains which under certain circumstances can compensate

for the shift in the position of Pro from S_3 to S_4. The substrate specificity of SCD towards Dns-PLALWAR and Dns-PLALFAR which cleaves two positions is very unique compared to the other MMPs. The matrilysin cleaved both substrates exclusively between A and L[5]. This phenomenon is in good agreement that human stromelysin-1 and neutrophil collagenase have different S_1' pocket with fibroblast collagenase and matrilysin[30]. The former group consists of a shallow pocket where the bottom is defined by the bulky and large side chains, whereas the latter group consists of a deep hydrophobic pocket. Our results confirmed the differences of the S_1' pocket by showing that SCD has broad P_1' residues. The single cleavage of Dns-PLALRAR and Dns-PLELFAR implies that the charged amino acids Arg and Glu are not favorable to the S_1' and S_2 sites, respectively, whereas Trp and Phe as well as Leu are adaptable to the S_1' pocket. It is consistent with the finding that MMPs generally prefer hydrophobic residues in the S_1' and S_2 sites[24,25]. Our results clearly demonstrate the importance of the peptide sequence for *in vitro* study of stromelysin-1. A more detailed analysis for mapping the subsite specificity, using a large number of different peptides, would be necessary to differentiate the physiological role and understand the structure-function relationship among MMPs.

References

1. Birkedal-Hansen, H., W. G. I. Moore, M. K. Bodden, L. J. Windsor, B. Birkedal-Hansen, A. DeCarlo and J. A. Engler. 1993. Matrix metalloproteinases: A review. *Crit. Rev. Oral Biol. Med.* **4**, 197-250.
2. Bond, M. D., D. S. Auld and R. R. Lobb. 1986. A convenient fluorescent assay for vertebrate collagenases. *Anal Biochem.* **155**, 315-321.
3. Cha, J. 1999. Mutational Analysis of two conserved active site tyrosine residues in matrilysin. *J. Life Sci.* **9**, 44-48.
4. Cha, J. and D. S. Auld. 1997. Site-directed mutagenesis of the active site glutamate in human matrilysin: Investigation of its role in catalysis. *Biochemistry* **36**, 16019-16024.
5. Cha, J., M. V. Pedersen and D. S. Auld. 1996. Metal and pH dependence of heptapeptide catalysis by human matrilysin. *Biochemistry* **35**, 15831-15838.
6. Cha, J., M. V. Sørensen, Q.-Z. Ye and D. S. Auld. 1998. Selective replacement of the catalytic zinc of the human stromlyein-1 catalytic domain. *J. Biol. Inorg. Chem.* **3**, 353-359.
7. Christianson, D. W. and W. N. Lipscomb. 1989. Carboxypeptidase A. *Acc. Chem. Res.* **22**, 62-69.
8. Cheung, N. T., D. J. Taylor and P. T. Dawes. 1995. Elevated plasma stromelysin levels in arthritis. *J. Rheum.* **22**, 2004-2005.
9. Crabbe, T., F. Willenbrock, D. Eaton, P. Hynds, A. F. Carne, G. Murphy and A. J. P. Docherty. 1992. Biochemical characterization of matrilysin. activation conforms to the stepwise mechanisms proposed fro other matrix metalloproteinases. *Biochemistry* **31**, 8500-8507.
10. Dhanaraj, V., Q.-Z. Ye, L. L. Johnson, D. J. Hupe, D. F. Ortwine, J. B. Jr. Dunbar, J. R. Rubin, A. Pablovsky, C. Humblet and T. L. Blundell. 1996. X-ray structure of a hydroxamate inhibitor complex of stromelysin catalytic domain and its comparison with members of the zinc metalloproteinase superfamily. *Structure* **4**, 375-386.
11. Harrison, R. K., B. Chang, L. Niedzwiecki and R. L. Stein. 1992. Mechanistic studies on the human matrix metalloproteinase stromelysin. *Biochemistry* **31**, 10757-10762.
12. Holman, C. M., C.-C. Kan, M. R. Gehring and H. E. Van Wart. 1999. Role of His-224 in the anomalous pH dependence of human stromelysin-1. *Biochemistry* **38**, 677-681.
13. Imai, K., Y. Yokohama, I. Nakanishi, E. Ochuchi, Y. Fujii, N. Nakai and Y. Okada. 1995. Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. *J. Biol. Chem.* **270**, 6691-6697.
14. Knauper, V., S. M. Wilhelm, P. K. Seperack, Y. A. DeClerck, K. E. Langley, A. Osthues and H. Tschesche. 1993. Direct activation of human neutrophil procollagenase by recombinant stromelysin. *Biochem. J.* **295**, 581-586.
15. Kunugi, S., H. Hirohara and N. Ise. 1982. pH and

temperature dependences of thermolysin catalysis, catalytic role of zinc-coordinated water. *Eur. J. Biochem.* **224**, 157-163.

16. Li, J., P. Brick, M. C. O'Hare, T. Skarzynski, L. F. Lloyd, V. A. Curry, I. M. Clark, H. F. Bigg, B. L. Hazleman, T. E. Cawston and D. M. Blow. 1995. Structure of full-length porcine synovial collagenase reveals a C-terminal domain containing a calcium-linked, flour-bladed β-propeller. *Structure* **3**, 541-549.
17. Lipscomb, W. N. 1973. Enzymatic activities of carobxypeptidase A's in solution and in crystals. *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3797-3801.
18. Macdougal, J. R. and L. M. Matrisian. 1995. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion, and metastasis. *Cancer Metastasis Rev.* **14**, 351-362.
19. Manicourt, D., N. Fuhimoto, K. Obata and E. M. A. Thonar. 1995. Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. *Arthritis Rheumatism* **38**, 1031-1039.
20. Marcy, A. I., L. L. Eiberger, R. Harison, H. K. Chan, N. I. Hutchinson, W. K. Hagmann, P. M. Cameron, D. A. Boulton and J. D. hermes. 1991. Human fibroblast stromelysin catalytic domain: expression, purification, and characterization of a C-terminally truncated form. *Biochemistry* **30**, 6476-6483.
21. Matrisian, L. M. 1992. The matrix-degrading metalloproteinases. *Bioessays* **14**, 455-463.
22. Matthews, B. W. 1988. Structural basis of the action of thermolysin and related zinc peptidases. *Acc. Chem. Res.* **21**, 333-340.
23. Murphy, G., M. I. Cockett, P. E. Stephens, B. J. Smith and A. J. P Docherty. 1987. Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. *Biochem. J.* **248**, 265-268.
24. Netzel-Arnett, S., Q. Sang, W. G. I. Moore, M. Navre, H. Birkedal-Hansen and H. E. Van Wart. 1993. Comparative sequence specificities of human 72- and 92-kDa gelatinases (type IV collagenases) and PUMP (matrilysin). *Biochemistry* **32**, 6427-6432.
25. Niedzwiecki, L., J. Teahan, R. K. Harrison and R. L. Stein. 1992. Substrate specificity of the human matrix metalloproteinase stromelysin and the development of continuous fluorometric assays. *Biochemistry* **31**, 12618-12623.
26. Ng, M. and D. S. Auld. 1989. A fluorescent oligopeptide energy transfer assay with broad applications for neutral proteases. *Anal. Biochem.* **183**, 50-56.
27. Stack, M. S. and R. D. Gray. 1990. The effect of pH, temperature, and D_2O on the activity of porcine synovial collagenase and gelatinase. *Arch. Biochem. Biophys.* **281**, 257-263.
28. Stöcker, W., M. Ng and D. S. Auld. 1990. Fluorescent oligopeptide substrates for kinetic characterization of the specificity of *Astacus* protease. *Biochemistry* **29**, 10418-10425.
29. Tronrud, D. E., A. F. Monzingo and B. W. Matthews. 1986. Crystallographic structural analysis of phosphoramidates as inhibitors and transition-state analogs of thermolysin. *Eur. J. Biochem.* **157**, 261-268.
30. Welch, A. R., C. M. Holman, M. Huber, M. C. Brenner, M. F. Browner and H. E. Van Wart. 1996. Understanding the P_1' specificity of the matrix metalloproteinases: Effect of S_1' pocket mutations in matrilysin and stromelysin-1. *Biochemistry* **35**, 10103-10109.
31. Windsor, L. J., D. L. Steele, S. B. LeBlanc and K. B. Taylor. 1997. Catalytic domain comparisons of human fibroblast-type collagenase, stromelysin-1, and matrilysin. *Biochem. Biophys. Acta* **1334**, 261-272.
32. Ye, Q., L. L. Johnson, D. J. Hupe and V. Baragi. 1992. Purification and characterization of the human stromelysin catalytic domain expressed in *Escherichia coli*. *Biochemistry* **31**, 11231-11235.

초록 : Stromelysin-1에 의한 펩타이드 가수분해에서 pH와 기질특이성 연구

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Stromelysin-1 catalytic domain (SCD)의 pH 특성과 기질 특이성이 형광물질을 띠는 펩타이드를 기질로 이용하여 stopped-flow technique와 HPLC 방법으로 검사되었다. 합성펩타이드 기질에 대한 SCD의 pH profile은 pH 5.5 부근에서 최적 활성을 보였고 pH 7 부근에서 shoulder를 나타내었다. 위의 pH profile로부터 얻어진 pK_a 값은 각각 5.0, 5.7 그리고 9.8이었다. 이러한 pattern은 오직 두 pK_a 값만을 갖는 다른 MMP family의 pH profile과는 큰 차이를 보였다. 여러 기질에 대한 작용 특성 또한 TLC와 HPLC 방법으로 검사되었다. SCD는 Dns-PLALWAR과 Dns-PLALFAR 두 기질에 대하여 A-L 결합과 L-W/F 결합을 모두 공격한 반면, 다른 MMP family중의 하나인 matrilysin은 A-L 결합만을 공격하여 분해하였다. 이러한 두 다른 MMP의 기질에 대한 구별되는 작용특성은 3차구조로부터 밝혀진 active site에 위치한 S_1' pocket의 구조적 차이에 의한 것으로 추측된다. 이러한 결과로부터 여러 합성펩타이드를 이용하여 각각의 MMP들의 기질에 대한 작용특성과 생리학적인 기능에 대한 이해가 가능하리라 생각된다.