Volume 297, number 1,2, 119–123 FEBS 10640 February 1992

Substrate and inhibitor studies on proteinase 3

Chih-Min Kam[a], John E. Kerrigan[a], Koert M. Dolman[b], Roel Goldschmeding[b], Albert E.G.Kr. Von dem Borne[b] and James C. Powers[a]

[a]*School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA* and [b]*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Laboratory for Experimental and Clinical Immunology and Department of Hematology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

Received 21 November 1991

Various amino acid and peptide thioesters were tested as substrates for human proteinase 3 and the best substrate is Boc-Ala-Ala-Nva-SBzl with a k_{cat}/K_m value of 1.0×10^6 $M^{-1} \cdot s^{-1}$. Boc-Ala-Ala-AA-SBzl (AA = Val, Ala, or Met) are also good substrates with k_{cat}/K_m values of (1–4) $\times 10^5$ $M^{-1} \cdot s^{-1}$. Substituted isocoumarins are potent inhibitors of proteinase 3 and the best inhibitors are 7-amino-4-chloro-3-(2-bromoethoxy)isocoumarin and 3,4-dichloroisocoumarin (DCI) with $k_{obs}/[I]$ values of 4700 and 2600 $M^{-1} \cdot s^{-1}$, respectively. Substituted isocoumarins, peptide phosphonates and chloromethyl ketones inhibited proteinase 3 less potently than human neutrophil elastase (HNE) by 1–2 orders of magnitude.

Peptide chloromethyl ketone; Peptide phosphonate; Proteinase 3; Substituted isocoumarin; Peptide thioester

1. INTRODUCTION

Proteinase 3 is a serine protease in the azurophil granules of polymorphonuclear leukocytes (PMN) and is distinct from human neutrophil elastase (HNE) and cathepsin G [1]. Recently, proteinase 3 has been cloned [2] and found to be identical to the antimicrobial protein p29b/AGP7 [3–5] and to myeloblastin, a serine protease derived from HL-60 leukemia cells [6,7]. Proteinase 3 is at least as prominent in neutrophil azurophilic granules as HNE [2]. The active sites of both enzymes are very similar [5]. Proteinase 3 degrades elastin [8], induces emphysema when administered to hamsters by intratracheal instillation [1] and serves as an antigen recognized by Wegener's Granulomatosis autoantibodies [8–11]. Proteinase 3 has also been found to degrade a variety of other matrix proteins [12]. We report here the reactivity of proteinase 3 with various peptide thioester substrates to further elucidate the substrate reactivity of proteinase 3, and the development of inhibitors which may be useful for elucidating the function of proteinase 3 in vitro and in vivo.

Correspondence address: J.C. Powers, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA. Fax: (1) (404) 894 7452.

Abbreviations: Abu, α-aminobutanoic acid; Boc, *t*-butyloxycarbonyl; 3-BrEtOIC, 3-(2-bromoethoxy)isocoumarin; DCI, 3,4-dichloroisocoumarin; DFP, diisopropyl fluorophosphate; 3-EtOIC, 3-ethoxyisocoumarin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; HNE, human neutrophil elastase; MeO, methoxy; Nva, Norvaline; PIPES, piperazine-*N*,*N*-bis(2-ethanesulfonic acid); PMSF, phenylmethanesulfonyl fluoride; 3-PrOIC, 3-propoxyisocoumarin; *p*NA, *p*-nitroanilide; PPE, porcine pancreatic elastase; ONp, *p*-nitrophenylester; SBzl, thiobenzyl ester.

2. EXPERIMENTAL

Percoll was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Triton X-100 was purchased from Sigma Chemical Co., St. Louis, MO. Adenosine triphosphate (ATP) was obtained from Boehringer, Mannheim, Germany. ^{125}I-Na was obtained from the Radiochemical Centre, Amersham, UK. Matrex Gel orange A was purchased from Amicon Div., Danvers, MA. Bio-Rex 70 was obtained from Bio-Rad Laboratories, Richmond, CA. XAR5 film was purchased from Eastman Kodak Co., Rochester, NY. Purified human neutrophil elastase (HNE) was obtained from Elastin Products, Pacific, MO and from the research laboratory of Dr. James Travis, University of Georgia. EDTA was purchased from Aldrich Chemie, Brussels, Belgium. Sheep anti-serum against cathepsin G was obtained from ICN Biochemicals, Lisle, IL. HEPES was obtained from Research Organics Inc., Cleveland, OH. 4,4′-Dithiodipyridine was purchased from Aldrich Chemical Co., Milwaukee, WI. All the peptide thioesters were prepared as previously described [13,14]. Substituted isocoumarins [15–17], peptide phosphonates [18], and peptide chloromethyl ketones [19] were prepared previously.

2.1. *Preparation of proteinase 3*

Blood from healthy donors was anticoagulated with trisodium citrate. Mononuclear cells and platelets were removed by centrifugation over isotonic Percoll with a specific gravity of 1.076 g/ml (1,000 × *g*, 20 min, 20°C). Erythrocytes were subsequently lysed with NH_4Cl (155 mM), $KHCO_3$ (10 mM), EDTA (10 μM) at 4°C. Thereafter, the neutrophils were washed twice with PBS/0.1% (v/v) BSA and finally resuspended in medium that consisted of 100 mM KCl, 3 mM NaCl, 1 mM ATP, 3.5 mM $MgCl_2$, 10 mM PIPES, pH 7.3. More than 95% of the cells were neutrophils.

Purified neutrophils, 75×10^9 in 300 ml of medium were nitrogen cavitated [20]. Nuclei and unbroken cells were pelleted by centrifugation at 500 × *g* for 10 min at 4°C. The supernatant was collected and centrifuged for 30 min at 35,000 × *g* at 4°C. The pellet containing the mixed granules fractions was resuspended in 0.08 M citrate phosphate

buffer pH 3.0/0.1% (v/v) Triton X-100 and sonicated at 45 kHz for three periods of 10 s each. Thereafter, soluble and insoluble materials were separated by centrifugation at 220,000 × g for 1 h at 4°C and the supernatant was stored at −70°C.

Proteinase 3 was purified from crude granule extract by dye–ligand affinity chromatography over Matrex Gel orange A followed by ion-exchange chromatography over Bio-Rex 70, as described previously [1]. Proteinase 3 was detected by hydrolysis of α-naphthyl acetate and by ELISA using a MAb 12.8 against proteinase 3. The fractions containing proteinase 3 were pooled and concentrated by ultrafiltration using an Amicon unit (filtration cut off 10,000 Da) and dialyzed against PBS. Contamination with HNE and cathepsin G in the proteinase 3 preparation was excluded by ELISA's [9], using MAb NP 57 (anti-NE) [21] and sheep anti-serum against cathepsin G. Purity was further proven by the absence of contaminants in SDS-PAGE analysis with subsequent silver staining and autoradiography after radiolabeling (Fig. 1). Protein concentration was measured by the Bradford technique, using a BSA standard. A total of 1.5 mg of proteinase 3 was purified from approximately 150 mg of crude granule extract. Aliquots of 500 μg/ml were stored at −70°C.

2.2. *Substrate kinetics*

The enzymatic hydrolysis of peptide thioester substrates catalyzed by human proteinase 3 was measured in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer containing 9% Me_2SO and at 25°C in the presence of 4,4'-dithiodipyridine [22]. All stock solutions of substrate were prepared in Me_2SO and stored at −20°C. The initial rates were measured at 324 nm (ε_{324} = 19800 $M^{-1} \cdot cm^{-1}$) using a Beckman 35 spectrophotometer when a 10–25 μl of an enzyme stock solution was added to a cuvette containing 2.0 ml of buffer, 150 μl of 4,4'-dithiodipyridine (5 mM) and 25 μl of substrate. The same volume of substrate and 4,4'-dithiodipyridine was added to the reference cell in order to compensate for the background hydrolysis rate of the substrates. Initial rates were measured in duplicate for each substrate concentration and were averaged in each case. The kinetic constants k_{cat}, K_m and k_{cat}/K_m were obtained from Lineweaver–Burke plots which had correlation coefficients greater than 0.99. For Z-Ala-SBzl, the plot went through the origin and only k_{cat}/K_m could be determined.

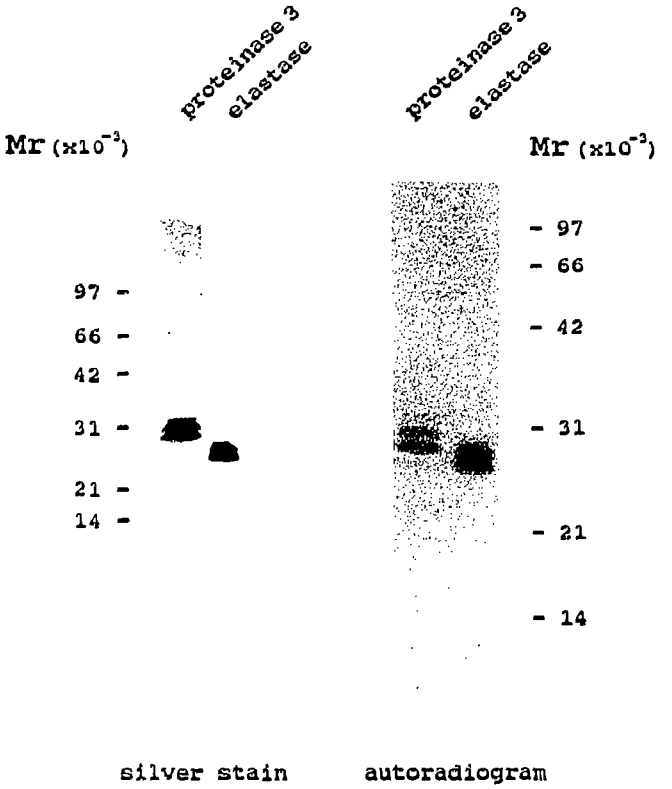

Fig. 1. SDS-PAGE analysis of proteinase 3 and HNE. Purified proteins (200 ng per lane) were applied on a 10–15% gradient gel under non-reducing conditions and stained with silver after separation. ^{125}I-labeled proteins (10 ng per lane) were applied on a 12% gel under non-reducing conditions and visualized by autoradiography after separation. Proteinase 3 appeared to be pure and exhibited an electrophoretic mobility different from HNE.

2.3. *Enzyme inactivation – incubation method*

An aliquot of inhibitor (25 μl) in Me_2SO was added to 0.25 ml of a buffered enzyme solution (0.13 μM) containing albumin (10–25 μl of 0.1 g/ml) to initiate the inactivation. Aliquots (50 μl) were withdrawn at various intervals and the residual enzymatic activity was measured as described above using Suc-Ala-Ala-Nva-SBzl (88 μM) as the substrate. The inhibitor concentrations are shown in Table II. Pseudo first-order inactivation rate constants (k_{obs}) were obtained from plots of ln v_i/v_o vs. time, and the correlation coefficients were greater than 0.98.

3. RESULTS AND DISCUSSION

3.1. *Substrate kinetics*

The substrate activity of proteinase 3 has been studied previously with oxidized insulin chains, and chromogenic and fluorogenic substrates [12]. The cleavage of insulin chains by proteinase 3 suggests that this enzyme preferentially attacks peptide bonds having small aliphatic amino acids such as Ala, Val, Leu and Ser at the P_1 and P_1' sites. The hydrolysis of synthetic peptides also indicates proteinase 3 prefers small aliphatic residues at the P_1 site and Pro at the P_2 site. Proteinase 3 did not hydrolyze HNE substrates such as Suc-Ala-Ala-Ala-*p*NA, and Suc-Ala-Ala-Val-*p*NA, but hydrolyzed Boc-Val-ONp, Boc-Ala-ONp, MeO-Suc-Ala-Ala-Pro-Val-*p*NA, and MeO-Suc-Ala-Ile-Pro-Met-*p*NA. We have tested amino acid and peptide thioesters including Boc-Ala-Ala-AA-SBzl (AA = Ala, Abu, Gly, Arg, Asp, Asn, Met, Met(O), Pro, Nva, Val, Ile, Leu, Nle, Phe, and Ser) as substrates of proteinase 3. The best substrates are tripeptide thioesters with Nva in the P_1 site. Z-Ala-SBzl, Boc-Ala-Ala-Ala-SBzl, Suc-Ala-Ala-Met-SBzl, Boc-Ala-Ala-Met-SBzl, Boc-Ala-Ala-Abu-SBzl, Boc-Ala-Ala-Val-SBzl, and MeO-Suc-Ala-Ala-Pro-Val-SBzl are also good substrates. All other thioesters show little activity. The kinetic constants k_{cat}, K_m and k_{cat}/K_m of several better substrates for proteinase 3 and HNE are shown in Table I. The kinetic constants of these thioester substrates for HNE have been reported previously [13] and are shown here for comparison with proteinase 3. The best substrate is Boc-Ala-Ala-Nva-SBzl with k_{cat}/K_m value of 1.0×10^6 $M^{-1} \cdot s^{-1}$ which is ca. 9-fold lower than the value of HNE. Suc-Ala-Ala-Nva-SBzl is the only substrate which has a higher k_{cat}/K_m value for proteinase 3 than for HNE. Substitution of Boc with Suc in Boc-Ala-Ala-Met-SBzl and Boc-Ala-Ala-Nva-SBzl slightly reduced the k_{cat}/K_m values for proteinase 3 by 1.5- to 2.1-fold, however this substitution resulted in a 50-fold reduction

Table I

Kinetic constants for hydrolysis of thioester substrates by proteinase 3 and human leukocyte elastase (HNE)[a]

Substrates		HNE[b]	Proteinase 3
Boc-Ala-Ala-Ala-SBzl	k_{cat}	63	10.1
	K_m	87	106
	k_{cat}/K_m	0.73	0.095
Boc-Ala-Ala-Val-SBzl	k_{cat}	9.0	10.4
	K_m	3.0	28
	k_{cat}/K_m	3.0	0.38
Boc-Ala-Ala-Nva-SBzl	k_{cat}	20	63.3
	K_m	2.2	63
	k_{cat}/K_m	9.2	1.0
Suc-Ala-Ala-Nva-SBzl	k_{cat}	110	89.7
	K_m	590	192
	k_{cat}/K_m	0.18	0.47
Boc-Ala-Ala-Met-SBzl	k_{cat}	82	12.2
	K_m	96	61
	k_{cat}/K_m	0.86	0.2
Suc-Ala-Ala-Met-SBzl	k_{cat}		19.0
	K_m		148
	k_{cat}/K_m		0.13
Boc-Ala-Ala-Ile-SBzl	k_{cat}	5.4	1.9
	K_m	1.4	31
	k_{cat}/K_m	3.9	0.06
Z-Ala-SBzl	k_{cat}		–
	K_m		–
	k_{cat}/K_m		0.11

[a]Kinetic constants were measured in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer, 9% Me_2SO and at 25°C. The units for k_{cat}, K_m and k_{cat}/K_m were s^{-1}, μM, and 10^6 $M^{-1} \cdot s^{-1}$.
[b]Data obtained from reference [13].

in the k_{cat}/K_m values for HNE. This indicates that P_3 site of proteinase 3 is more polar than that of HNE. Boc-Ala-Ala-Ile-SBzl had a 65-fold lower k_{cat}/K_m value for proteinase 3 than for HNE and tripeptide thioesters which contain Leu or Nle at P_1 site were poor substrates of proteinase 3. The partial primary structure of proteinase 3 has been determined [12] and comparison of active site region of proteinase 3 with HNE indicates that a strong homology exists between these two enzymes. However, there are differences in several residues in the primary binding pocket S_1. For example, Val-190 and Ala-213 of HNE which are located at the bottom of S_1 subsite [23] are replaced by Ile and Asp in proteinase 3 [12], and these changes may explain the differences in the enzymatic reactivities of proteinase 3 and HNE. Our studies on thioester substrates indicate that proteinase 3 prefers smaller aliphatic residues such as Ala, Val, and Nva at the P_1 site, while HNE can tolerate larger side chains such as Ile, Leu, or Nle [13].

3.2. *Inhibitor kinetics*

The esterolytic activity of proteinase 3 was inhibited by PMSF and DFP [1]. Proteinase 3 was not inhibited by the PPE inhibitor MeO-Suc-Ala-Ala-Pro-Ala-CH_2Cl, however the HNE inhibitor MeO-Suc-Ala-Ala-Pro-Val-CH_2Cl showed weak inhibition toward this enzyme with $k_{obs}/[I]$ of 10 $M^{-1} \cdot s^{-1}$ [12]. Elafin, a human skin inhibitor of HNE was found to be a potent inhibitor of proteinase 3 with IC_{50} of 9.5 nM [24]. A series of succinimidyl sulfonate derivatives have been reported to be potent irreversible inhibitors of proteinase 3, although their inhibition mechanism is not yet known [25]. We tested substituted isocoumarins, peptide phosphonates (structures shown in Fig. 2), and peptide chloromethyl ketones as inhibitors of proteinase 3 and HNE, and the inhibition constants are shown in Table II. 3,4-Dichloroisocoumarin, a general serine protease inhibitor, inhibited proteinase 3 quite potently with a $k_{obs}/[I]$ of 2,600 $M^{-1} \cdot s^{-1}$, which is ca. 3.5-fold lower than the value for HNE. The inhibitory potency of 3-alkoxy-7-amino-4-chloroisocoumarins toward proteinase 3 depends on the nature of the alkoxy group. A 3-bromoethoxy isocoumarin inhibited proteinase 3 ca. 6- to 8-fold better than the corresponding 3-ethoxy and 3-propoxy derivatives. This trend is also observed with HNE. This may indicate that 3-bromoethoxyisocoumarin binds to the enzyme differently from 3-ethoxy and 3-propoxyisocoumarins. The X-ray crystal structure of PEE and 7-amino-3-bromoethoxy-4-chloroisocoumarin has been solved recently [17]. The bromoethoxy group H-bonds with Gln-192 and goes toward the S_3 subsite instead of binding to the S_1 subsite as is likely for the other alkoxyisocoumarins. Although the 7-acetylamino and 7-phenylcarbamoylamino isocoumarin derivatives inhibited proteinase 3 more potently than 7-amino-4-chloro-3-propoxyisocoumarin by 3-fold, all the 7-substituted-3-bromoethoxy-4-chloroisocoumarins with bulky aromatic groups inhibited proteinase 3 less potently than the parent 7-amino compound. Substitution with bulky groups at the 7-position

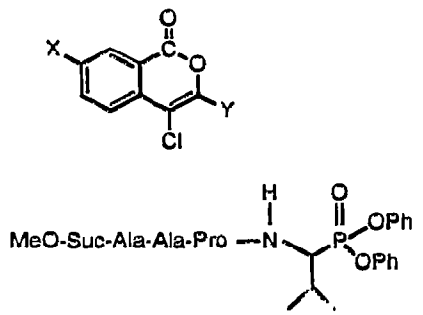

Fig. 2. Structures of substituted isocoumarins (top) and a peptide phosphonate. 3,4-Dichloroisocoumarin, X = H, Y = Cl; 7-amino-3-(2-bromoethoxy)-4-chloroisocoumarin, X = NH_2, Y = OCH_2CH_2Br; 4-chloro-7-phenylcarbamoylamino-3-propoxyisocoumarin, X = PhNHCONH, Y = $OCH_2CH_2CH_3$. A peptide phosphonate MeO-Suc-Ala-Ala-Pro-Val^P$(OPh)_2$ is shown at the bottom of the figure.

Table II

Inhibition constants of proteinase 3 and HNE by substituted isocoumarins, peptide phosphonates and peptide chloromethyl ketones[a]

Inhibitors	Proteinase 3		HNE	
	[I] (μM)	k_{obs}/[I] ($M^{-1} \cdot s^{-1}$)	[I] (μM)	k_{obs}/[I] ($M^{-1} \cdot s^{-1}$)
3,4-dichloroisocoumarin	3.6	2,600	1.1	8,900[b]
7-NH_2-4-Cl-3-EtOIC	40.7	560	4	9,400[c]
7-NH_2-4-Cl-3-PrOIC	38	740	2.6	54,000
7-NH_2-4-Cl-3-BrEtOIC	7.3	4,700	0.07	200,000
7-CH_3CONH-4-Cl-3-PrOIC	7.7	2,200	1.0	>190,000
7-PhNHCONH-4-Cl-3-PrOIC	7.7	1,700	1.2	140,000
7-*S*-(Naphthyl)(CH_3)CHNHCONH-4-Cl-3-BrEtOIC	6.8	2,100 310	0.44	310,000
7-*R*-(Naphthyl)(CH_3)CHNHCONH-4-Cl-3-BrEtOIC	6.2	1,800 330	0.64	170,000
7-*S*-PhNHCOOCH(CH_3)CONH-4-Cl-3-BrEtOIC	7.4	2,000 300	0.8	140,000
MeO-Suc-Ala-Ala-Ala-$Nva^P(OPh)_2$	427	3.3		
MeO-Suc-Ala-Ala-Ala-$Val^P(OPh)_2$	426	30	5	1,500[d]
MeO-Suc-Ala-Ala-Pro-$Nva^P(OPh)_2$	427	21	95	380[d]
MeO-Suc-Ala-Ala-Pro-$Val^P(OPh)_2$	78	150	4.9	7,100[d]
Boc-Val-Pro-$Val^P(OPh)_2$	323	46	4.5	27,000[d]
MeO-Suc-Ala-Ala-Pro-Val-CH_2Cl	400	5.9		1,600
Boc-Ala-Ala-Pro-Val-CH_2Cl	416	1.4		
Z-Gly-Leu-Ala-CH_2Cl	400	14%[e]		20[f]
Ac-Ala-Ala-Ala-CH_2Cl	395	11%[e]		2.8[f]
Ac-Ala-Ala-Pro-Ala-CH_2Cl	400	6%[e]		44[f]

[a] Inhibition constants were measured in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer, 9% Me_2SO, 0.3 mg of albumin and at 25°C. Suc-Ala-Ala-Nva-SBzl (88 μM) was used as the substrate.
[b] Data obtained from reference [15].
[c] Data obtained from reference [16].
[d] Data obtained from reference [18].
[e] Percentage of inhibition was obtained after 10 min of incubation.
[f] Data obtained from reference [27].

of 7-amino-4-chloro-3-propoxyisocoumarin generally improves the inhibitory potency toward HNE by 3- to 4-fold, whereas the same substitution in 7-amino-3-bromoethoxy-4-chloroisocoumarin did not change the inhibitory potency significantly. These 7-acylamino groups are directed toward the S′ subsites [26] which indicates that proteinase 3 and HNE must have significant difference in this region of the active site. It has been shown that Phe-41 which is part of the S_1' and S_3' subsites in HNE remains unchanged in proteinase 3 [12,23]. However, in this region there are two insertions in proteinase 3 when compared with HNE, a two amino acid insertion between Gly-38 and Gly-39, and a single amino acid insertion between Gly-39 and His-40. These insertions may account for the reactivity differences between proteinase 3 and HNE toward acylaminoisocoumarins. The inhibition reaction of 7-substituted-3-bromoethoxy-4-chloro-isocoumarin towards proteinase 3 was biphasic and the rate constants were based on the 40–50% inhibition observed during the first 1–2 min incubation.

Peptide phosphonates are good inhibitors of HNE [18], but did not inhibit proteinase 3 very well. The best inhibitor is MeO-Suc-Ala-Ala-Pro-$Val^P(OPh)_2$ (k_{obs}/[I] = 27,000 $M^{-1} \cdot s^{-1}$) which inhibits proteinase 3 ca. 50-fold less potently than HNE. The best HNE inhibitor Boc-Val-Pro-$Val^P(OPh)_2$ inhibits proteinase 3 with k_{obs}/[I] of 50 $M^{-1} \cdot s^{-1}$. As previously described, all the peptide chloromethyl ketones which we tested also inhibited proteinase 3 poorly. Roe et al. [12] found no inhibition of proteinase 3 by MeO-Suc-Ala-Ala-Pro-Ala-CH_2Cl.

In summary, we have found that peptide thioesters are good substrates of proteinase 3 and substituted isocoumarins inhibit this enzyme quite potently. Comparison of the activity of proteinase 3 and HNE indicates that there are significant differences in the extended substrate binding sites (S_4 subsite is more polar, S_1 and S′ subsites are more restrictive in proteinase 3) and the catalytic residues (proteinase 3 has lower reactivity toward most substrates and inhibitors). Thioester substrates and isocoumarin inhibitors should be useful for future studies on the functional role of proteinase 3.

Acknowledgements: This research was supported by grants to the Georgia Institute of Technology (HL34035 and HL29307) from the National Institutes of Health and to the University of Amsterdam from Institutes of Health and from the Dutch Kidney Foundation (c

88.733). The authors would like to thank Dr. Jozef Oleksyszyn for preparing several isocoumarin and phosphonate derivatives.

REFERENCES

[1] Kao, R.C., Wehner, N.G., Skubitz, K.M., Gray, B.H. and Hoidal, J.R. (1988) J. Clin. Invest. 82, 1963–1973.
[2] Campanelli, D., Melchior, M., Fu, Y., Nakata, M., Shuman, H., Nathan, C.F. and Gabay, J.E. (1990) J. Exp. Med. 172, 1709–1715.
[3] Gabay, J.E., Scott, R.W., Campanelli, D., Griffith, J., Wilde, C., Marra, M.N., Seyer, M. and Nathan, C.F. (1989) Proc. Natl. Acad. Sci. USA 86, 5610–5614.
[4] Campanelli, D., Detmers, P.A., Nathan, C.F. and Gabay, J.E. (1990) J. Clin. Invest. 85, 904–915.
[5] Wilde, C.G., Snable, J.L., Griffith, J.E. and Scott, R.W. (1991) J. Biol. Chem. 265, 2038–2041.
[6] Bories, D., Raynal, M.C., Soloman, D.H., Darzynkiewicz, Z. and Cayre, Y.E. (1989) Cell 59, 959–968.
[7] Jenne, D.E., Tschopp, J., Lüdemann, J., Utecht, B. and Gross, W.L. (1990) Nature 346, 520.
[8] Lüdemann, J., Utecht, B. and Gross, W.L. (1990) J. Exp. Med. 171, 357–362.
[9] Goldschmeding, R., Van der Schoot, C.E., Ten Bokkel Huinink, D., Hack, C.E., Van den Ende, M.E., Kallenberg, C.G.M. and Von dem Borne, A.E.G.Kr. (1989) J. Clin. Invest. 84, 1577–1587.
[10] Niles, J.L., McCluskey, R.T., Ahmad, M.F. and Arnaout, M.A. (1989) Blood 74, 1888–1893.
[11] Jennette, J.C., Holidal, J.R. and Falk, R.J. (1990) Blood 75, 2263–2265.
[12] Rao, N.V., Wehner, N.G., Marshall, B.C., Gray, W.R., Gray, B.H. and Hoidal, J.R. (1991) J. Biol. Chem. 266, 9540–9548.
[13] Harper, J.W., Cook, R.R., Roberts, J., McLaughlin, B.J. and Powers, J.C. (1984) Biochemistry 23, 2995–3002.
[14] McRae, B.J., Kurachi, K., Heimark, R.L., Fujikawa, K., Davie, E.W. and Powers, J.C. (1981) Biochemistry 20, 7196–7206.
[15] Harper, J.W., Hemmi, K. and Powers, J.C. (1985) Biochemistry 24, 1831–1841.
[16] Harper, J.W. and Powers, J.C. (1985) Biochemistry 24, 7200–7213.
[17] Vijayalakshmi, J., Meyer Jr., E.F., Kam, C.-M. and Powers, J.C. (1990) Biochemistry 30, 2175–2183.
[18] Oleksyszyn, J. and Powers, J.C. (1991) Biochemistry 30, 485–493.
[19] Powers, J.C., Gupton, B.F., Harley, A.D., Nishino, N. and Whitley, R.J. (1977) Biochim. Biophys. Acta 485, 158–166.
[20] Borregaard, N., Heiple, J.M., Simons, F.R. and Clark, R.A. (1983) J. Cell Biol. 97, 52–61.
[21] Pulford, K.A.F., Erber, W.N., Crick, J.A., Olsson, I., Micklem, K.J., Gatter, K.C. and Mason, D.Y. (1988) J. Clin. Pathol. 41, 853–860.
[22] Grasetti, D.R. and Murray, J.F. (1967) Arch. Biochem. Biophys. 119, 41–49.
[23] Bode, W., Meyer Jr., E. and Powers, J.C. (1989) Biochemistry 28, 1951–1963.
[24] Wiedow, O., Lüdemann, J. and Utecht, B. (1991) Biochem. Biophys. Res. Commun. 174, 6–10.
[25] Groutas, W.C., Hoidal, J.R., Brubaker, M.J., Stanga, M.A., Venkataraman, R., Gray, B.H. and Rao, N.V. (1990) J. Med. Chem. 33, 1087–1090.
[26] Hernandez, M.A., Powers, J.C., Glinski, J., Oleksyszyn, J., Vijayalakshmi, J. and Meyer Jr., E.F. (1991) J. Med. Chem., in press.
[27] Tuhy, P.M. and Powers, J.C. (1975) FEBS Lett. 50, 359–361.