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Compared Action of Neutrophil Proteinase 3 and Elastase on Model Substrates

FAVORABLE EFFECT OF S′-P′ INTERACTIONS ON PROTEINASE 3 CATALYSIS*

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Neutrophil proteinase 3 (Pr3) and elastase (NE) may cause lung tissue destruction in emphysema and cystic fibrosis. These serine proteinases have similar P_1 specificities. We have compared their catalytic activity using acyl-tetrapeptide-*p*-nitroanilides, which occupy the S_5-S'_1 subsites of their substrate binding site, and intramolecularly quenched fluorogenic heptapeptides, which bind at S_5-S'_4. Most *p*-nitroanilide substrates are turned over slowly by Pr3 as compared with NE. These differences disappear with the fluorogenic heptapeptides, some of which are hydrolyzed even faster by Pr3 than by NE. Elongation of substrates strongly increases the catalytic efficiency of Pr3, whereas it has little effect on NE catalysis. These different sensitivities to S′-P′ interactions show that Pr3 and NE are not interchangeable enzymes despite their similar P_1 specificity.

The azurophilic granules of polymorphonuclear neutrophils contain three serine proteinases: elastase (NE),[1] cathepsin G, and proteinase 3 (Pr3), which participate in lysosomal bacterial digestion and neutrophil migration through the extracellular matrix at sites of inflammation. These enzymes are ~30-kDa glycoproteins, which belong to the chymotrypsin family of serine proteinases. Pr3 is the most recently discovered, the most difficult to isolate, and hence the less well studied proteinase of the three. It is identical to three independently discovered proteins: (i) myeloblastin, which regulates the growth and differentiation of leukemic cells; (ii) p29b, which has microbicidal activity; and (iii) the target antigen of antineutrophil cytoplasmic autoantibodies detected in patients with Wegner's granulomatosis (2). Pr3 cleaves extracellular matrix proteins including elastin, type IV collagen, fibronectin, laminin, and vitronectin (3, 4). It is able to produce lung emphysema in hamsters (3), and thus, in concert with NE and cathepsin G, it may be responsible for lung tissue destruction occurring in emphysema and cystic fibrosis.

Although many model substrates have been used to map the active site of NE (5–8), literature on the substrate specificity and the catalytic activity of Pr3 is poorly documented. Fuginaga *et al.* (9) have shown that the two enzymes have similar substrate binding sites. This explains why both proteinases cleave the oxidized insulin A and B chains at peptide bonds involving small aliphatic amino acid residues (4). The specificity of Pr3 for non-bulky residues has been confirmed with a limited number of model substrates (4, 10–12). As a rule, these substrates were turned over at a much lower rate by Pr3 than by NE, an unexplained observation. We have undertaken the present work to understand this puzzling finding.

EXPERIMENTAL PROCEDURES

Materials—NE was isolated, and active site was titrated as described previously (13). Pr3 came from Athens Research Technology (Athens, GA) and was titrated with α_1-proteinase inhibitor (4). This enzyme preparation was electrophoretically pure. The presence of NE was tested by reacting 0.14 μM Pr3 with increasing amounts of secretory leukoprotease inhibitor (1–8 μM, final concentration), which inhibits NE but does not inhibit Pr3 (3). The buffer was the same as that used for enzyme kinetics. After 5 min at 25 °C, 2.1 mM MeOSuc-Ala_2-Pro-Val-*p*NA, a very sensitive NE substrate (5), was added to the mixtures and the enzymic rates were measured at 410 nm and 25 °C. The lack of enzyme activity indicated that this commercial preparation of Pr3 was free of contaminating NE. Most *p*-nitroanilide substrates were purchased from Bachem (Bubendorf, Switzerland). Suc-Ala_2-Asp-Val-*p*NA, Suc-Ala_2-Glu-Val-*p*NA, Suc-Leu-Val-Glu-Ala-*p*NA, Suc-Ala_4-*p*NA, MeOSuc-L2p-Tyr-Asp-Ala-*p*NA, and MeOSuc-L2p-Tyr-Asp-Val-*p*NA were synthesized by Enzyme System Products (Livermore, CA). The intramolecularly quenched fluorogenic substrates Mca-peptide-Dpa-NH_2 were synthesized using the same methodology as described earlier (14). The compounds were purified by reverse-phase chromatography, and their identity was confirmed by mass spectrometry. All of the substrates were dissolved in dimethylformamide. Their concentration was checked spectrophotometrically using $\epsilon_{315\ nm}$ = 14,600 M^{-1} cm^{-1} and $\epsilon_{410\ nm}$ = 7,500 M^{-1} cm^{-1} for *p*-nitroanilides and fluorescent substrates, respectively.

Kinetics of Substrate Hydrolysis—All of the kinetic measurements were done at 25 °C and pH 7.4 (50 mM Hepes, 150 mM NaCl). The enzymatic reactions were initiated by adding a small volume of enzyme solution to the buffered substrate solution contained in a spectrophotometer or a fluorometer cuvette. The final concentration of dimethylformamide was 5% (v/v) throughout.

The initial rate of *p*-nitroanilide hydrolysis was measured at 410 nm using variable concentrations of substrate and constant concentrations of enzyme. For the most sensitive substrates, the final Pr3 and NE concentrations were 140 and 60 nM, respectively. For the less sensitive substrates, 10–50-fold higher enzyme concentrations were used. The kinetic parameters k_{cat} and K_m were calculated from non-linear least square fits to the Michaelis-Menten equation (Enzfitter software). The Pr3-catalyzed hydrolysis of Suc-Ala_4-*p*NA was so slow that k_{cat} and K_m could not be determined separately. Therefore, we recorded the full hydrolysis of 10–30 μM Suc-Ala_4-*p*NA by 3.3 μM Pr3. The k_{cat}/K_m ratio for this enzyme-substrate pair was calculated from the progress curve as outlined below for the fluorogenic substrates.

The cleavage of the fluorogenic substrates was monitored at λ_{ex} =

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[1] The abbreviations used are: NE, human neutrophil elastase; Pr3, human neutrophil proteinase 3; Suc, succinyl; MeOSuc, methoxysuccinyl; Ac, acetyl; Boc, *tert*-butyloxycarbonyl; *p*NA, *p*-nitroanilide; L2p, lysyl-(2-picolinoyl); Mca, (7-methoxycoumarin-4-yl)acetyl; Dpa, *N*-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl; nb, number.

TABLE I

Kinetics of the hydrolysis of acyl-tetrapeptide p-nitroanilides by Pr3 and NE at pH 7.4 and 25 °C

For the sake of clarity, the kinetic constants are rounded off and the errors attached to them are not given. The errors on k_{cat} and K_m were equal or lower than 14 and 19%, respectively. ND, not determined.

Substrate							Enzyme	k_{cat}	K_m	k_{cat}/K_m
Structure										
nb	P_5	P_4	P_3	P_2	P_1	P'_1				
								s^{-1}	mM	$M^{-1}s^{-1}$
1	Suc-	Ala-	Ala-	Ala-	*p*NA		Pr3	ND	ND	9
							NE	0.45	0.13	3,460
2	Ac-	Ala-	Ala-	Pro-	Ala-	*p*NA	Pr3	0.22	2.7	80
							NE	4.7	1.7	2,800
3	Suc-	Ala-	Ala-	Pro-	Ala-	*p*NA	Pr3	0.23	1.9	120
							NE	10.0	1.35	7,400
4	Boc-	Ala-	Ala-	Pro-	Ala-	*p*NA	Pr3	0.5	2.00	250
							NE	3.4	0.35	9,700
5	Suc-	Ala-	Ala-	Pro-	Abu-	*p*NA	Pr3	0.3	0.38	790
							NE	9.1	0.57	15,800
6	Ac-	Ala-	Ala-	Pro-	Val-	*p*NA	Pr3	1.0	2.8	360
							NE	8.3	0.46	18,000
7	Suc-	Ala-	Ala-	Pro-	Val-	*p*NA	Pr3	0.2	0.6	360
							NE	10.4	0.13	77,200
8	MeOSuc-	Ala-	Ala-	Pro-	Val-	*p*NA	Pr3	0.5	0.47	1,060
							NE	10.6	0.1	107,000
9	Suc-	Ala-	Ala-	Pro-	Nva-	*p*NA	Pr3	0.09	0.5	180
							NE	6.9	1.2	5,700
10	Suc-	Ala-	Ala-	Pro-	Met-	*p*NA	Pr3		not hydrolyzed	
							NE	0.33	2.5	130
11	Suc-	Ala-	Ala-	Pro-	Leu-	*p*NA	Pr3		not hydrolyzed	
							NE	0.3	1.6	190
12	Suc-	Ala-	Ala-	Pro-	Ile-	*p*NA	Pr3	0.27	0.64	420
							NE	0.41	0.46	860
13	Suc-	Ala-	Ala-	Pro-	Nle-	*p*NA	Pr3		not hydrolyzed	
							NE	0.3	2.5	120
14	Suc-	Leu-	Val-	Glu-	Ala-	*p*NA	Pr3	0.37	0.90	400
							NE	0.43	0.65	680
15	Suc-	Ala-	Ala-	Asp-	Val-	*p*NA	Pr3	1.4	0.62	2,300
							NE	0.9	0.75	1,200
16	Suc-	Ala-	Ala-	Glu-	Val-	*p*NA	Pr3	1.0	0.97	1,020
							NE	5.8	0.83	7,000
17	Suc-	Ala-	Tyr-	Leu-	Val-	*p*NA	Pr3	0.6	0.17	3,500
							NE	2.7	0.09	28,900
18	MeOSuc-	L2p-	Ala-	Pro-	Val-	*p*NA	Pr3[a]	2.3	0.14	16,300
							NE	1.5	0.03	54,300
19	MeOSuc-	L2p-	Tyr-	Asp-	Ala-	*p*NA	Pr3	3.2	0.10	32,000
							NE	0.9	0.13	6,800
20	MeOSuc-	L2p-	Tyr-	Asp-	Val-	*p*NA	Pr3	2.4	0.013	184,600
							NE	11.0	0.13	84,600

[a] k_{cat} = 0.49 s^{-1}, K_m = 0.06 mM, k_{cat}/K_m = 30,600 M^{-1} s^{-1} in 50 mM phosphate buffer, pH 7.0, containing 0.05% Triton X-100 and 0.5% dimethylsulfoxide (12).

328 nm and λ_{em} = 393 nm (14) using substrate concentrations below 10 μM to avoid absorptive fluorescence quenching of the hydrolysis product. The absorptive quenching also precluded the separate measurement of k_{cat} and K_m. Therefore, the hydrolysis of the fluorogenic substrates was followed to completion. At low substrate concentrations ($[S]_o < K_m$), the Michaelis-Menten equation simplifies to $v = k_{cat}/K_m \times [E]_o \times [S]_o$ so that the release of product P with time is a first-order reaction described by $d[P]/dt = k_{obs}[S]$ where $k_{obs} = k_{cat}/K_m \times [E]_o$. The pseudo first-order rate constant k_{obs} was calculated by non-linear regression analysis of progress curves recorded using 0.5–5 μM substrate concentrations and 0.1–1 μM enzyme concentrations. The progress curves were all first-order, and k_{obs} did not significantly change with $[S]_o$. This indicates that $[S]_o$ was indeed lower than K_m.

Site of Substrate Cleavage—The *p*-nitroanilide substrates (2 μM) dissolved in the above buffer + 5% dimethylformamide were reacted with 0.3–15 μM enzyme in a total volume of 1 ml. After ~20% substrate hydrolysis, the reaction was stopped with 10 μl of trifluoroacetic acid, the medium was diluted 200-fold with buffer A (buffer + 0.1% trifluoroacetic acid), and 40 μl of this dilution was applied to a 3.9 × 100-mm C18 Novo-pack column equilibrated with buffer A. A linear gradient formed with buffers A and B (0.1% trifluoroacetic acid in acetonitrile) was used to separate the reaction products. Elution was followed at 214 nm at a flow rate of 0.8 ml·min^{-1}.

The fluorogenic substrates (5 μM) dissolved in the buffer + 5% dimethylformamide were reacted with 30 nM to 1.6 μM enzyme in a total volume of 1 ml. After ~50% hydrolysis, the reaction was stopped with 30 ml of glacial acetic acid. The digests were absorbed on C18 reverse-phase cartridges, washed with 5% acetonitrile to remove the salts, and then the peptide fragments eluted with 60% acetonitrile. The eluant mixtures were rotary-evaporated to dryness and taken up in 0.1 ml of 60% acetonitrile. The fragments were identified by electrospray ionization mass spectrometry. The fragment signals were 10–100 times that of the background.

RESULTS

Compared Action of Pr3 and NE on Acyl-tetrapeptide p-Nitroanilides—All of the substrates listed in Table I are hydrolyzed more or less efficiently by NE, but many of them are more resistant to Pr3 than to NE. The proteolytic coefficient k_{cat}/K_m may be up to 380-fold lower for Pr3 than for NE (see substrate **1**). For compound **8**, the best NE substrate (5), the difference is 100-fold. With a few exceptions, the differences in reactivity are because of differences in k_{cat}. Very poor NE substrates are not hydrolyzed at all by Pr3 (see nb **10**, **11**, **13**). Also, none of the substrates Suc-Ala-Ala-Pro-*X*-pNA (*X* = Asp, Glu, Lys, and ornithine) is hydrolyzed by either NE or Pr3. The poor catalytic power of Pr3 is not the result of non-productive enzyme-substrate binding because high pressure liquid chromatography analyses showed that the only reaction products resulting from partial hydrolysis by Pr3 (or NE) were acyl-tetrapeptides +

TABLE II

Compared catalytic activity of Pr3 and NE on intramolecularly quenched fluorogenic substrates at pH 7.4 and 25 °C

The error on k_{cat}/K_m is ≤ 20%. The table also compares fluorogenic (Fluo) and *p*-nitroanilide substrates (from Table I) encompassing identical N-terminal tetrapeptidic sequences. Fluo/*p*NA means k_{cat}/K_m of fluorogenic substrate/k_{cat}/K_m of *p*NA substrates. The arrow indicates the cleavage points. n.h. = no hydrolysis.

Substrates		Pr3		NE	
nb	Structures	k_{cat}/K_m	Fluo/*p*NA	k_{cat}/K_m	Fluo/*p*NA
	↓	$M^{-1}\ s^{-1}$		$M^{1}\ s^{-1}$	
21	Mca-Ala-Ala-Pro-Leu-Lys-Gly-Asp-Dpa NH_2	115	∞	1,200	6.3
11	Suc-Ala-Ala-Pro-Leu-*p*Na	n.h.		190	
22	Mca-Ala-Ala-Ala-Ala-Lys-Gly-Asp-Dpa-NH_2	2,600	290	5,000	1.4
1	Suc-Ala-Ala-Ala-Ala-*p*NA	9		3,500	
23	Mca-Ala-Ala-Pro-Val-Lys-Gly-Asp-Dpa-NH_2	62,000	172	179,000	2.3
7	Suc-Ala-Ala-Pro-Val-*pNA*	360		77,000	
24	Mca-L2p-Tyr-Asp-Ala-Lys-Gly-Asp-Dpa-NH_2	1,100,000	34	2,300	0.34
19	MeOSuc-L2p-Tyr-Asp-Ala-*p*NA	32,000		6,800	
25	Mca-L2p-Tyr-Asp-Val-Lys-Gly-Asp-Dpa-NH_2	4,600,000	25	88,000	1.04
20	MeOSuc-L2p-Tyr-Asp-Val-*p*NA	184,600		84,600	
26	Mca-L2p-Tyr-Asp-Ile-Lys-Gly-Asp-Dpa-NH_2	910,000		95,000	
27	Mca-L2p-Val-Glu-Ala-Lys-Gly-Asp-Dpa-NH_2	610,000		55,000	

p-nitroaniline, which indicates that binding involves subsites S_5 to $S'1$[2] of the active center.

A number of compounds are cleaved by Pr3 and NE with comparable proteolytic coefficients. These are either poor (nb **12**, **14**, **15**) or good NE substrates (nb **18**-**20**). Compound **19** is the only *p*-nitroanilide whose k_{cat} for Pr3 is significantly higher than that for NE.

Compared Action of Pr3 and NE on Fluorogenic Peptides—We have synthesized a number of heptapeptides to cover both S and S′ subsites of the active center of Pr3 and NE. To follow their hydrolysis using fluorescence emission, we have included the fluorescent label Mca and the fluorescence quencher Dpa (14) at their N and C termini, respectively. For reasons given under "Experimental Procedures," k_{cat} and K_m could not be determined separately. However, their ratio is meaningful because (i) it represents the second-order acylation rate constant whether acylation is rate-limiting or not and whether substrate binding is productive or not (15), and because (ii) it is a good measure of specificity (16).

The cleavage sites of the fluorogenic substrates were determined by electrospray ionization mass spectrometry. The masses (Da) of the N-terminal peptides generated by Pr3 and NE were 609.5 and 609.3 for substrate 21 (theoretical mass of Mca-Ala-Ala-Pro-Leu = 609.7); 547.1 and 545 for substrate 22 (Mca-Ala_4 = 541.5); 839.7 and 839.4 for substrate 24 (Mca-L2p-Tyr-Asp-Ala = 839.8); 867.7 and 865.5 for substrate 25 (Mca-L2p-Tyr-Asp-Val = 867.9); 880.8 and 879.5 for substrate 26 (Mca-L2p-Tyr-Asp-Ile = 881.9); and 789.3 and 788.2 for substrate 27 (Mca-L2p-Val-Glu-Ala = 789.8). The N-terminal peptide generated by the action of NE on substrate 23 had a mass of 595.6 (Mca-Ala_2-Pro-Val = 596.6). The Pr3 + substrate 23 mixture did not give a clear-cut mass profile because of paucity of the material.

Table II shows that Pr3 hydrolyzes most fluorogenic substrates very rapidly. It is even more effective than NE on compounds 24–27. Table II also compares the proteolytic coefficients of Pr3 and NE on six heptapeptidic and tetrapeptidic substrates encompassing identical N-terminal tetrapeptidic sequences. It can be seen that the catalytic activity of Pr3 dramatically increases with peptide chain elongation, whereas that of NE does not significantly vary with substrate length.

DISCUSSION

Pr3 and NE belong to the chymotrypsin-like family of serine proteinases whose active site is composed of a substrate-binding site responsible for specificity and of a catalytic site responsible for substrate hydrolysis. The latter comprises a highly conserved Asp-His-Ser triad whose hydrogen bonding transforms the serine Oγ into a powerful nucleophile that attacks the scissile peptide bond of the substrate. The efficacy of catalysis is due in part to a precise binding of substrate with resultant proper orientation of the scissile bond with respect to Oγ of the catalytic serine (17). Fuginaga *et al.* (9) have shown that Pr3 and NE have similar substrate-binding sites that account for their preference for small aliphatic residues at P_1 (4). However, the use of some model substrates and inhibitors indicated that Pr3 is a much poorer catalyst than NE (4, 10–12). We thought this might be because of the fact that Pr3 (but not NE) binds these compounds in a predominantly non-productive way. Therefore, we used a series of acyl-tetrapeptide-*p*-nitroanilides, which all bind productively at subsites S_5 to S'_1. This series included substrates with P_2 = Asp or Glu to explore the possibility of electrostatic S_2-P_2 interactions (9). Because the catalytic differences persisted for most of these compounds, we hypothesized that the occupancy of subsites S_5 to S'_1 of Pr3 may not be sufficient to align the substrate in a position favorable for efficient catalysis, whereas it may be sufficient in the case of NE. The use of fluorogenic heptapeptides confirmed our hypothesis that elongation of the substrates strongly increased the catalytic efficiency of Pr3, whereas it had a much less effect on NE catalysis. Thus, the poor catalytic activity of Pr3 on tetrapeptidic substrates is no longer observed with heptapeptidic substrates, some of which are turned over even more rapidly by Pr3 than by NE. It is interesting to note that the rate-enhancing effect attributed to full occupancy of the S′ region of Pr3 is more pronounced for poor *p*-nitroanilide substrates (nb **1, 7, 11** with P_4 = Ala) than for good ones (nb **19, 20** with P_4 = L2p). This finding suggests that S_4-P_4 and S′-P′ interactions play a complementary role in binding substrates nb **19** and **20** in a position favorable for catalysis.

[2] The S and S′ subsites of the substrate binding site and the corresponding P and P′ residues of the substrate are labeled according to the nomenclature of Schechter and Berger (1).

Whereas many studies have probed the S region of the substrate binding site of serine proteinases, literature is poorly documented on the S′-binding region (19). Stein and Strimpler (7) have shown that the favorable effect of S′-P′ interaction on NE catalysis (18) is mainly the result of occupancy of subsite S'_1 because there are no important binding interactions available past S'_1. This is in agreement with our data showing that elongation of the NE substrates beyond S'_1 has no significant effect on catalysis.

The present work is the first report on S′-P′ interactions in Pr3. It explains why *p*-nitroanilides (4, 12) and thiobenzyl esters (10, 11) were found to be poor substrates of Pr3. It also allows the conclusion that Pr3 and NE cannot be considered as two interchangeable enzymes despite their common specificity for small aliphatic amino acid residues. Indeed, the divergent effects of S′-P′ binding may lead to different specificities on biological protein substrates. In this context, it is noteworthy that Pr3 and NE show differences in the digestion pattern of fibronectin, laminin, and collagen (4).

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REFERENCES

1. Schechter, I., and Berger, A. (1967) *Biochem. Biophys. Res. Commun.* **27,** 157–162
2. Hoidal, J. R. (1998) in *Handbook of Proteolytic Enzymes* (Barrett, A. J., Rawlings, N. D., and Woessner, J. F., Jr., eds) pp. 62–65, Academic Press, London, United Kingdom
3. Kao, R. C., Wehner, N. G., Skubitz, K. M., Gray, B. H., and Hoidal, J. R. (1988) *J. Clin. Invest.* **82,** 1963–1973
4. Rao, N., Wehner, N., Marshall, B., Gray, W., Gray, B., and Hoidal, J. (1991) *J. Biol. Chem.* **266,** 9540–9548
5. Nakajima, K., and Powers, J. C. (1979) *J. Biol. Chem.* **254,** 4027–4032
6. Yasutake, A., and Powers, J. C. (1981) *Biochemistry* **20,** 3675–3679
7. Stein, R. L., and Strimpler, A. M. (1987) *Biochemistry* **26,** 2238–2242
8. Lestienne, P., and Bieth, J. G. (1980) *J. Biol. Chem.* **255,** 9289–9294
9. Fujinaga, M., Chernaia, M. M., Halenbeck, R., Koths, K., and James, M. N. G. (1996) *J. Mol. Biol.* **261,** 267–278
10. Brubaker, M. J., Groutas, W. C., Hoidal, J. R., and Rao, N. V. (1992) *Biochem. Biophys. Res. Commun.* **188,** 1318–1324
11. Kam, C. M., Kerrigan, J. E., Dolman, K. M., Goldschmeding, R., Von dem Borne, A. E. G. Kr., and Powers, J. C. (1992) *FEBS Lett.* **297,** 119–123
12. Früh, H., Kostoulas, G., Michel, B. A., and Baici, A. (1996) *Biol. Chem.* **377,** 579–586
13. Boudier, C., and Bieth J. G. (2001) *Biochemistry* **40,** 9962–9967
14. Knight, C. G., Willenbrock, F., and Murphy, G. (1992) *FEBS Lett.* **296,** 263–266
15. Bender, M. L., and Kezdy, F. J. (1965) *Annu. Rev. Biochem.* **34,** 49–76
16. Fersht, A. R. (1974) *Proc. R. Soc. Lond. B* **187,** 397–407
17. Perona, J. J., and Craik, C. S. (1995) *Protein Sci.* **4,** 337–360
18. Mc Rae, B., Nakajima, K., Travis, J., and Powers, J. C. (1980) *Biochemistry* **19,** 3973–3978
19. Hedstrom, L. (2002) *Chem. Rev.* **102,** 4501–4524

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