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Specific Inhibition of Mature Fungal Serine Proteinases and Metalloproteinases by Their Propeptides

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The function of the long propeptides of fungal proteinases is not known. *Aspergillus fumigatus* produces a 33-kDa serine proteinase of the subtilisin family and a 42-kDa metalloproteinase of the thermolysin family. These extracellular enzymes are synthesized as preproenzymes containing large amino-terminal propeptides. Recombinant propeptides were produced in *Escherichia coli* as soluble fusion proteins with glutathione *S*-transferase or thioredoxin and purified by affinity chromatography. *A. fumigatus* serine proteinase propeptide competitively inhibited serine proteinase, with a K_i of 5.3×10^{-6} M, whereas a homologous serine proteinase from *A. flavus* was less strongly inhibited and subtilisin was not inhibited. Binding of metalloproteinase propeptide from *A. fumigatus* to the mature metalloenzyme was demonstrated. This propeptide strongly inhibited its mature enzyme, with a K_i of 3×10^{-9} M, whereas thermolysin and a metalloproteinase from *A. flavus* were not inhibited by this propeptide. Enzymatically inactive metalloproteinase propeptide complex could be completely activated by trypsin treatment. These results demonstrate that the propeptides of the fungal proteinases bind specifically and inhibit the respective mature enzymes, probably reflecting a biological role of keeping these extracellular enzymes inactive until secretion.

Many proteinases are synthesized as preproenzymes consisting of a typical signal peptide followed by the propeptide and the mature protein (1). Several functions have been proposed for the propeptide. The propeptide may be required for aiding the proper folding or secretion of the mature proteinase (7, 8, 28, 34), anchoring the proteinase to the membrane and/or maintaining the proteinase in an inactive state until it is released from the cell (33). In several cases, the propeptide plays multiple roles. For instance, the propeptide of a bacterial α-lytic proteinase was reported to be involved in the correct folding of mature protein in vivo and in vitro (2, 3, 29–31) and in the inhibition of the activity of the mature proteinase (2). The propeptides of a number of eukaryotic proteinases have been shown to act as strong inhibitors, but their role in folding has not yet been examined. For example, the propeptides of the aspartyl proteinases, pepsin and cathepsin D, inhibit the mature enzymes (11); the propeptide region of the metalloproteinase, carboxypeptidase A, strongly inhibits the mature carboxypeptidase A (27); and a 62-amino-acid pro region of a cysteine proteinase, cathepsin B, strongly inhibits cathepsin B (9). Propeptides of fungal proteinases have not been tested as inhibitors of the respective mature enzymes. During the last several years, extracellular proteinases from different fungal species have been cloned and characterized (10, 13–15, 20, 25, 26, 32, 33). Some of them have large propeptides that in some cases approach the size of the mature enzyme (15, 20, 32). However, little is known about their function. In this paper, we describe expression of propeptides of a serine proteinase and a metalloproteinase from *Aspergillus fumigatus* and demonstrate that these propeptides are highly selective inhibitors of their respective mature enzymes.

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MATERIALS AND METHODS

Proteinase activity and inhibition assays. Subtilisin A from *Bacillus licheniformis*, human leukocyte elastase, and the elastase substrate methoxysuccinyl (MeO Suc)-Ala-Ala-Pro-Val–*p*-nitroanilide (*p*NA) were obtained from Calbiochem. Thermolysin from *Bacillus thermoproteolyticus* and Suc-Ala-Ala-Pro-Leu-*p*NA were obtained from Sigma.

The *A. fumigatus* and *A. flavus* serine proteinases were purified as described previously (18). Assays for the fungal serine proteinase activity and subtilisin A were done with Suc-Ala-Ala-Pro-Leu-*p*NA (0.1 M in dimethyl sulfoxide) as a substrate. The reaction mixtures (1 ml) contained aliquots of enzymes and 0.5 mM substrate in 50 mM Tris-HCl (pH 8.0), and the rates were monitored by measuring the change in A_{410}. The human elastase assay was done in 50 mM Tris-HCl (pH 7.0) with 1 mM substrate, and activity was measured spectrophotometrically at 410 nm. The metalloproteinase (MEP42) from *A. fumigatus* was purified as described previously (21). The assay for this enzyme was done in 2 ml of 0.1 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.0) by adding 2 μl of the 10 mM 2-aminobenzoyl(Abz)-Ala-Ala-Phe-Phe-*p*NA in methanol and aliquots of enzyme. The fluorescence of the released product was measured with the excitation wavelength at 340 nm and the emission wavelength at 420 nm.

For the inhibition studies, enzymes were preincubated for 5 to 30 min with the inhibitor and then the enzyme activity assay was performed as described above. Inhibition was maximal with 5 min of preincubation; therefore, for all subsequent experiments, a 5-min incubation was used. The K_m values of the serine and metalloproteinases were determined with Suc-Ala-Ala-Pro-Leu-*p*NA and Abz-Ala-Ala-Phe-Phe-*p*NA as substrates, respectively. The K_m for the serine proteinase and metalloproteinase were 4×10^{-4} and 2×10^{-6} M, respectively. Competitive inhibition constants were determined from the Lineweaver-Burk plots obtained with different concentrations of the inhibitors. K_i for the tightly bound inhibitors were determined from the apparent inhibitor constants $K_{i(app)}$ by the method of Bieth (4), using the estimated propeptide content of 50% in the partially purified preparation (as judged from Coomassie blue staining).

Reactivation of the MEP42-propeptide complex by trypsin treatment was determined by measuring enzyme activity after a 20-min treatment of the complex with 5 μg of trypsin. *Streptomyces* metalloproteinases inhibitor (SMPI) was kindly provided by K. Oda (Kyoto Institute of Technology, Kyoto, Japan). Protein was measured by the method of Bradford (6).

Expression of preprometalloproteinase, antibody production, and immunoblot. The prepro-MEP42 was obtained by expression of the open reading frame of the cDNA in *Escherichia coli* with the pET21b vector. MEP42 cDNA (32) was amplified with *A. fumigatus* cDNA as template with the following primers: sense, 5′-CGCGGCATATGCGCGGGCTTCTTCTGGC-3′, and antisense, 5′-GCGC GAGATCTTCAACAGACACCACTGGGG-3′. These primers introduced a *Nde*I site and a *Bgl*II site at the 5′ and 3′ ends, respectively. The amplified cDNA was cloned into vector pCR^{II}, and the clones with correct insertion and reading frame were identified by sequencing. The *Nde*I-*Bgl*II fragment was excised from pCR^{II} and cloned into the *Nde*I-*Bam*HI-digested pET21b vector (Novagen). Expression of the recombinant protein in *E. coli* BL21(DE3)(pLysS) was per-

formed as described for the expression of the mature recombinant enzyme (32). Rabbit polyclonal antibodies against prepro-MEP42 were prepared by using as an antigen the protein band cut out from the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. Immunoblotting was done as described previously (21).

Expression of the MEP42 propeptide in *E. coli*. The propeptide of the *A. fumigatus* metalloproteinase was expressed in *E. coli* with the putative 18-amino-acid signal sequence. To clone the propeptide coding region, we used PCR with metalloproteinase cDNA (32) as template and the following primers introducing *Nde*I and *Eco*RI sites at 5′ and 3′ ends, respectively: sense, 5′-CGCGGCATAT GCGCGGGCTTCTTCTGC-3′; and antisense, 5′-GGCGGAATTCTTCAGCA ACATAGTCCAC-3′. The amplified product was cloned into the pCRII vector, and the clones with correct insertion were identified by sequencing. The *Nde*I-*Eco*RI fragment was excised from pCR^{II} and cloned into the *Nde*I-*Eco*RI-digested pET21b vector (Novagen). Expression of the recombinant protein in *E. coli* BL21(DE3)(pLysS) was performed as described previously (32).

Affinity purification of the propeptide. *E. coli* cells harvested after induction were resuspended in 4 ml of binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl [pH 7.9]). Cells were lysed by sonication, and the lysate was subjected to centrifugation at 40,000 × *g* for 20 min at 4°C. The pellet was solubilized by being dispersed in 5 ml of binding buffer containing 6 M urea and stirred for 30 min. After the insoluble material was removed by centrifugation, the supernatant protein solution was applied to a His Bind column (3 ml) prepared as described by the manufacturer (Novagen), except that all buffers contained 6 M urea. To remove the urea, the protein was subjected to gel filtration on Sephadex G-25 equilibrated with 0.05 M HEPES (pH 7.0). The purity of the eluted protein was analyzed by SDS-PAGE (19).

Expression of the metalloproteinase propeptide fused with thioredoxin in *E. coli*. The *A. fumigatus* metalloproteinase propeptide sequence (32) was amplified with primers designed to remove the signal sequence and introduce *Xba*I and *Pst*I sites at the 5′ and 3′ ends, respectively, and cloned into pCR^{II} vector. The primers were 5′-GGTCTAGACCATCCCGCTCACCAG-3′ and 5′-GGCTGC AGTCATTCAGCAACATAG-3′. The *Xba*I-*Pst*I fragment was excised from the pCR^{II} vector and ligated into the *Xba*I-*Pst*I-digested pTrx Fus vector (ThioFusion Expression System; Invitrogen) in frame with the thioredoxin gene. After transformation, the cells were grown in RM medium (ThioFusion Expression System) with 100 μg of carbenicillin per ml at 30°C. After a 4-h induction with tryptophan (100 μg/ml) the cells were harvested by centrifugation and resuspended in 1 ml of buffer A (0.1 M HEPES [pH 7.0] containing 1 μg of leupeptin per ml). After mild sonication (three or four times for 10 s on ice at output 4 [Branson Sonifier 450]) and centrifugation at 10,000 × *g* for 15 min, 1 ml of the supernatant was mixed with 1 ml of ThioBond resin (Invitrogen) and stirred at room temperature for 60 min, and the mixture was transferred to a 0.8- by 4-cm column. The resin was washed with buffer A containing 1 mM 2-mercaptoethanol and subsequently with the same buffer containing 10, 50, and 200 mM 2-mercaptoethanol. The fractions were examined by SDS-PAGE.

Expression of the serine proteinase propeptide in *E. coli*. The cDNA for serine proteinase (18) and the following primers were used to PCR amplify the propeptide region: sense, 5′-GGGGGATCCCCGTCTTTGGCCCTCTC-3′; antisense, 5′-GGGGGATCCAAGGTACCAGATTTGGTC-3′. A *Bam*HI site in the sense primer and an *Eco*RI site in the antisense primer were introduced for subcloning. The cDNA corresponding to the propeptide was amplified by PCR, and the amplified products were cloned into the pCR^{II} vector. The *Bam*HI-*Eco*RI fragment was excised from pCR^{II} and ligated with pGEX-5X-3 expression vector (Pharmacia) digested with *Bam*HI-*Eco*RI. The vector contains the glutathione *S*-transferase (GST) gene in frame with the insert so that the propeptide would be expressed as a fusion with the C terminus of GST under the *tac* promoter. *E. coli* BL21 was transformed with the expression construct, and transformants were grown in Luria-Bertani broth containing ampicillin (100 μg/ml) at 37°C in a shaking incubator. After a 4-h induction with 1 mM IPTG, cells were harvested, resuspended in 1/50 culture volume of MTPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4 [pH 7.3]), and, after addition of Triton X-100 (1%), lysed by mild sonication (three or four times for 10 s each at output 4 on ice), and the lysate was subjected to centrifugation at 12,000 × *g* for 10 min at 4°C. The supernatant was mixed with 1 ml of glutathione-agarose beads (50% in MTPBS [Sigma]) for 2 to 5 min at room temperature, and the beads were pelleted by brief centrifugation at 500 × *g* and washed three times with MTPBS. Fusion protein was eluted with 1 bed volume of 5 mM reduced glutathione (Sigma) in 50 mM Tris-HCl (pH 8.0). The purity of the eluted fusion protein was analyzed by SDS-PAGE.

RESULTS

Inhibition of *A. fumigatus* metalloproteinase by its propeptide. To test whether the propeptide of *A. fumigatus* metalloproteinase (MEP42) could inhibit the mature enzyme, we expressed the 245-amino-acid propeptide (including the signal) in *E. coli* expression vector pET21b. Since the protein expressed even at 30°C was found in inclusion bodies (data not shown), the prepropeptide was solubilized in urea and purified by His Bind metal chelation chromatography. On SDS-PAGE, this preparation showed a 29-kDa major band which cross-reacted with rabbit antibodies prepared against the intact precursor protein obtained by expressing the cDNA in *E. coli* (data not shown). This peptide containing the signal peptide showed incomplete (60%) inhibition, presumably because of faulty refolding. To overcome these problems, we resorted to expression of the propeptide (without signal peptide) as fusion proteins. In an attempt to prepare recombinant protein in soluble form, the 227-amino-acid propeptide (without signal) was cloned in frame in pTrxFus, expression in *E. coli* was investigated at different growth temperatures, and thioredoxin-propeptide fusion protein induced with tryptophan at 30°C was released by osmotic shock. This propeptide-enriched preparation, as well as *E. coli* lysate containing this propeptide, inhibited the metalloproteinase activity in a concentration-dependent manner, whereas the control lysate without the propeptide did not inhibit the enzyme (Fig. 1). On the other hand, the propeptide did not strongly inhibit thermolysin (Fig. 1B), indicating that the inhibition by the propeptide is specific for its mature enzyme.

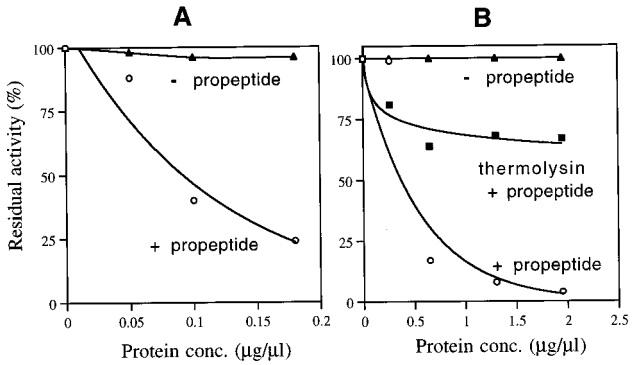

FIG. 1. Concentration-dependent inhibition of the *A. fumigatus* MEP42 and thermolysin by the propeptide of MEP42 fused to thioredoxin expressed in *E. coli*. MEP42 was incubated with different concentrations of shock fluid (A) and *E. coli* lysate (B) at room temperature for 30 min, and residual enzymatic activity was measured with the fluorigenic substrate Abz-Ala-Ala-Phe-Phe-*p*NA.

To determine the K_i of the MEP42 inhibition by the propeptide, the fusion protein was first purified from the high-speed supernatant of the cell lysate with ThioBond resin. SDS-PAGE of the protein eluted with 10 mM 2-mercaptoethanol showed a major protein band at 40 kDa (Fig. 2A). This partially purified propeptide inhibited the mature enzyme, and the K_i was 3×10^{-9} M (Fig. 3, inset), while the K_m for this enzyme was 2×10^{-6} M. Inhibitory activities of the propeptide toward MEP42 were measured at various molar ratios of inhibitor to proteinase with Abz-Ala-Ala-Phe-Phe-*p*NA as a substrate. The propeptide exhibited strong inhibitory activity toward MEP42, with a molar ratio of inhibitor to protease of 1 (Fig. 3). MEP42 inhibition by the propeptide was even stronger than that observed for the only known metalloproteinase inhibitor, SMPI. This extracellular protein of 102 amino acids, produced by *Streptomyces nigrescens* TK24, specifically inhibits several metalloproteinases such as thermolysin (23). We have found that SMPI inhibited MEP42 with a K_i of 4.6×10^{-9} M (unpublished data), which is close to the K_i for the propeptide inhibition. This result is not surprising, because the fungal metalloproteinase contains some of the conserved zinc-binding and active-site motifs characteristic of thermolysin and because the peptide bond cleavage specificity of the fungal enzyme showed similarities to that of the thermolysin (21, 32). On the other hand, the failure of the propeptide to inhibit thermolysin indicates that the propeptide is a more highly selective inhibitor than SMPI.

To test for binding of the propeptide to the mature enzyme, the cell extract containing the propeptide was subjected to

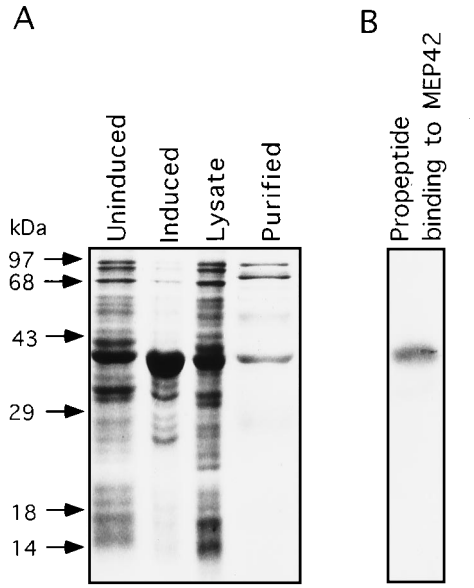

FIG. 2. (A) SDS-PAGE of the *A. fumigatus* MEP42 propeptide fused with thioredoxin expressed in *E. coli*. Uninduced, total proteins in the cell before induction with tryptophan; Induced, cells after a 4-h induction with tryptophan at 30°C; Lysate, soluble fraction from total-cell lysate; Purified, propeptide from affinity chromatography on ThioBond resin. (B) MEP42 binding to the propeptide. Samples of the fusion protein (150 μg) expressed in *E. coli* were subjected to SDS-PAGE and electrotransferred to a polyvinylidene difluoride membrane. After blocking with 0.1% Tween 20–5% skim milk, the membrane was incubated (2 h at room temperature) with or without MEP42 (3 μg/ml) and subsequently probed with antiMEP42 antibodies, horseradish peroxidase-conjugated protein A, and the enhanced chemiluminescence detection system as before. Panel B shows the signal with MEP42; without MEP42, no signal was detected.

SDS-PAGE followed by electroblotting to a polyvinylidene difluoride membrane. After blocking, the membrane was incubated with MEP42 and subsequently probed with antibodies against the mature enzyme. Blots were visualized by using horseradish peroxidase-protein A and the enhanced chemiluminescence detection system. A single band that cross-reacted with anti-MEP42 antibodies was seen at 40 kDa, exactly corresponding to the propeptide-thioredoxin protein (Fig. 2B). Without preincubation with MEP42, no band was seen on the blot (data not shown). These results demonstrate that the recombinant propeptide binds to the mature protein.

If the propeptide remained bound to the mature enzyme after cleavage of the propeptide, the enzyme would remain inactive and its activation might involve proteolytic destruction of the inhibitory propeptide. To test for this possibility, the effect of trypsin treatment on the enzymatic activity of the mature enzyme-propeptide complex was determined (Table 1). Inhibition of MEP42 by the propeptide was completely reversed by treatment of the complex with trypsin. Thus, proteolytic destruction of the propeptide is probably involved in the processing and activation of the metalloproteinase.

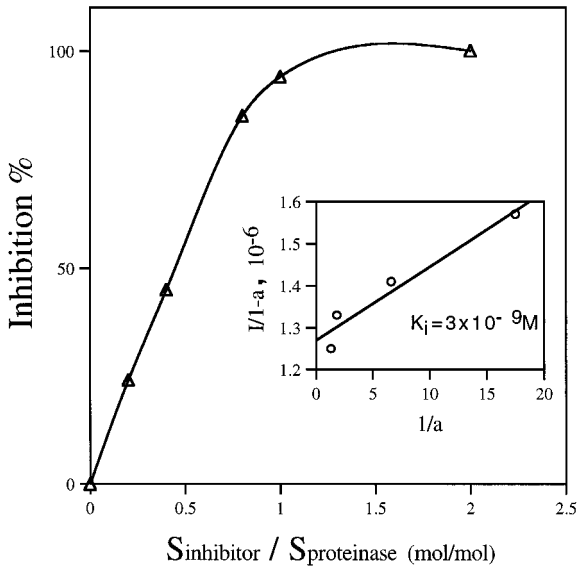

FIG. 3. Inhibition of MEP42 by thioredoxin-propeptide. Inhibition was measured with a fluorigenic substrate as indicated for Fig. 2. Inset, determination of the inhibitor constant by the method of Bieth (4).

Inhibition of the *A. fumigatus* serine proteinase by its propeptide. To test whether the propeptide of the serine proteinase can inhibit the mature enzyme, the propeptide was expressed in *E. coli*. The 103 amino acids corresponding to the propeptide sequence was PCR amplified and fused to the C-terminal end of GST in frame. When the production of the fusion protein was induced by isopropyl-β-D-thiogalactopyranoside (IPTG), the GST-propeptide produced was soluble, and this protein was the major protein band revealed by SDS-PAGE of the cell lysate (Fig. 4A). The fusion protein was purified by glutathione-agarose chromatography (Fig. 4B).

The purified GST-propeptide inhibited the mature enzyme in a concentration-dependent manner, whereas GST alone had no effect on the activity (Fig. 5A). The propeptide behaved as a competitive inhibitor of the serine proteinase (Fig. 5B). The inhibition by the propeptide was highly specific among closely related serine proteinases (Fig. 6). The propeptide strongly inhibited the mature serine proteinase, whereas a homologous serine proteinase from a related fungal species, *A. flavus*, was less strongly inhibited (Fig. 6). Even though these serine proteinases belong to the subtilisin family, the propeptide did not inhibit subtilisin itself.

DISCUSSION

In this paper, we show that propeptides of serine proteinase and metalloproteinase of *A. fumigatus* are inhibitors of their respective mature enzymes. For inhibition studies, we used

TABLE 1. Trypsin-mediated reversal of inhibition of *A. fumigatus* MEP42 by the propeptide-thioredoxin fusion protein[a]

Addition	Activity (%)
MEP42	100
Trypsin	0
MEP42 + trypsin incubated for 20 min	115
MEP42 + propeptide-thioredoxin incubated for 5 min	3
MEP42 + propeptide-thioredoxin incubated for 5 min followed by a 10-min incubation with trypsin	105

[a] Bovine trypsin (5 μg), propeptide (0.21 μg), and MEP42 (0.06 μg) were used in a total volume of 15 μl. Trypsin did not cleave the Abz-Ala-Ala-Phe-Phe-*p*NA used in the activity assay and had no effect on the activity of MEP42.

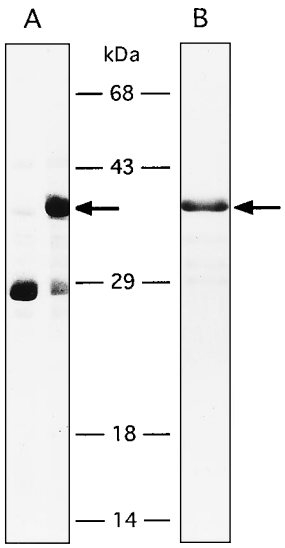

FIG. 4. (A) SDS-PAGE of *E. coli* cell lysate containing GST (left lane) or GST fused to the propeptide of *A. fumigatus* serine proteinase (right lane). The arrow indicates the expected size of the fusion protein. (B) GST fused to propeptide purified with glutathione-agarose beads.

recombinant propeptides expressed in *E. coli* as fusion proteins. A similar approach was used for producing propeptides of α-lytic protease and subtilisin (2, 24). The degree of inhibition of mature proteinases by their respective propeptides varies from moderate to strong. The MEP42 propeptide exhibited strong inhibition toward mature metalloproteinase, with a molar ratio of inhibitor to proteinase of 1:1 and inhibitor constant K_i of 3×10^{-9} M. The propeptide of another known metalloproteinase, porcine carboxypeptidase A, was also shown to be a powerful inhibitor of the active enzyme with a K_i in the nanomolar range (27), and carboxypeptidase A also formed a 1:1 molar complex with its propeptide (27). Recent studies on one of the best-characterized metalloproteinases, *Pseudomonas aeruginosa* elastase, demonstrated strong propeptide binding and inhibition of mature elastase (17). *A. fumigatus* serine proteinase inhibition by its propeptide was weaker, with an inhibitor constant of about 5.3×10^{-6} M. This value was similar to the inhibition constant of the propeptide of subtilisin for its mature enzyme (24). On the other hand, the propeptide of the extracellular bacterial serine proteinase, α-lytic protease, strongly inhibited the mature enzyme, with a K_i of close to 10^{-11} M (2).

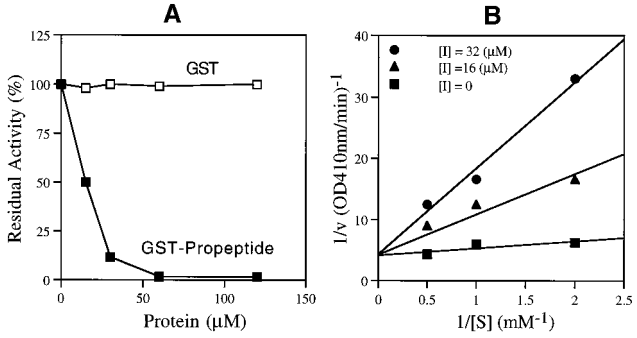

FIG. 5. Inhibition of the *A. fumigatus* serine proteinase by purified GST-serine proteinase propeptide. (A) Concentration dependence of serine proteinase inhibition. (B) Double-reciprocal plot showing competitive inhibition of the serine proteinase by the propeptide.

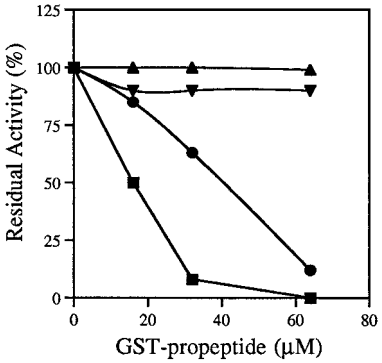

FIG. 6. Specificity of inhibition of proteinases by the GST-serine proteinase propeptide. Leukocyte elastase (▲) activity was measured with MeO-$(Ala)_2$-Pro-Val-*p*NA as the substrate, and the serine proteinases from *A. flavus* (●) and from *A. fumigatus* (■) and subtilisin (▼) were measured with Suc-Ala-Ala-Pro-Leu-*p*NA as the substrate.

Inhibition of the mature proteinase by their respective long propeptides showed a high degree of specificity. The inhibition of MEP42 by its propeptide was stronger than that observed with SMPI, which was previously isolated as a strong inhibitor of thermolysin (23). The K_i of SMPI for MEP42 was 4.6×10^{-9} M (unpublished results). On the other hand, the propeptide of MEP42 or purified propeptide-thioredoxin fusion protein had little effect on thermolysin activity. It also did not inhibit a different metalloproteinase (MEP20) from *A. flavus*, a closely related species. Even though inhibition of serine proteinase by its propeptide was less strong than that observed for metalloproteinase, the high degree of specificity of inhibition by the propeptide was clear with the serine proteinase. For example, *A. fumigatus* serine proteinase showed 83% sequence homology to the serine proteinase from *A. flavus* (25), but the inhibition of this enzyme by the *A. fumigatus* serine proteinase propeptide was much lower than that observed with the mature serine proteinase of *A. fumigatus*. The inhibition of the subtilisin A by the propeptide was insignificant, even though the catalytic motif of *A. fumigatus* serine proteinase is identical to that of subtilisin. This type of high specificity appears to be a common feature of inhibition of other mature proteinases by their propeptides. Thus, inhibition of elastase, subtilisin, α-lytic protease and carboxypeptidase A by their propeptides was found to be specific. The propeptide of α-lytic proteinase inhibits the closely related *Streptomyces griseus* proteinase B but not the more distantly related yet structurally similar mammalian pancreatic elastase (2). The propeptide of porcine carboxypeptidase A is inhibitory to its bovine counterpart but not to porcine carboxypeptidase B (27). In contrast, propeptides of aspartic proteinases showed a very broad inhibitory spectrum (11).

The large propeptide probably keeps MEP42 inactive until it is released from the cell. The cDNA sequence of MEP42 (32) indicates that this proteinase is synthesized as a 634-amino-acid preproenzyme containing a signal sequence of 18 amino acids and a 227-amino-acid propeptide. It is interesting that bacterial elastase and MEP42 propeptides constitute about 36% of their mature enzymes. The processing of MEP42 may involve a sequence of events somewhat analogous to that proposed for elastase processing (17). The signal peptide of the bacterial enzyme is removed as the protein passes through the inner membrane, and the resulting proenzyme is processed autocatalytically (16, 22). The cleaved propeptide remains noncovalently associated with the mature enzyme until secretion of mature enzyme through the outer membrane (17). The mechanisms underlying the final maturation step are poorly under-

stood. Our results that the propeptide-mature enzyme complex is enzymatically inactive until trypsin treatment, as previously observed with the bacterial α-lytic proteinase and elastase (2, 17), suggest that fungal MEP42 propeptide plays a role similar to that suggested for the bacterial propeptides. Our results show that trypsin degradation of the propeptide in the propeptide-mature enzyme complex restores full MEP42 activity. It is likely that in vivo, one or more of the many secreted proteinases are responsible for activating MEP42 by degrading the inhibitory propeptide. The propeptide of serine proteinase might play a similar role in processing.

In case of α-lytic proteinase and subtilisin E, propeptides have been shown to play a fundamental role in the folding of their native enzymes (12, 29, 30). Recent structural analysis showed that the free proregion of subtilisin is largely unstructured but when complexed with subtilisin, folds into a four-stranded antiparallel β-sheet and two three-turn α-helices. X-ray structure of the complex revealed a prodomain binding, suggesting that the prodomain catalyzes subtilisin folding by stabilizing the central αβα substructure of subtilisin (6a). In contrast to chaperonins showing low specificity and extremely weak affinity for native proteins (5), propeptides bind the folded proteinases with high affinity and specificity (2, 24). In this paper, we show that MEP42 propeptide binds with high affinity and specificity to its mature proteinase. This might be a characteristic of propeptides that also act as chaperonins, and the propeptide-assisted folding mechanism may be used by the fungal metalloproteinase.

The mechanism and structural basis for the inhibition of proteinases by their propeptides have not yet been elucidated. Crystal structures of proteinase-propeptide complexes have not been reported. However, inhibition of proteinases by specific binding of the cleaved propeptides, demonstrated here for the first time for fungal proteinases, appears to be a strategy shared by evolutionarily and mechanistically unrelated proteinases.

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