Hepatocyte growth factor activator inhibitor type 1 (HAI-1) is a Kunitz-type serine protease inhibitor identified as a strong inhibitor of hepatocyte growth factor (HGF) activator and matriptase. HAI-1 is first produced in a membrane-integrated form with two Kunitz domains in its extracellular region, and subsequent ectodomain shedding releases two major secreted forms, one with a single Kunitz domain and one with two Kunitz domains. To determine the roles of the Kunitz domains in the inhibitory activity of HAI-1 against serine proteases, we constructed various HAI-1 mutant proteins and examined their inhibitory activity against HGF activator and trypsin. The N-terminal Kunitz domain (Kunitz I) had potent inhibitory activity against both HGF activator and trypsin, whereas the C-terminal Kunitz domain (Kunitz II) had only very weak inhibitory activity against HGF activator, although its potency against trypsin was equivalent to that of Kunitz I. These results indicate that Kunitz I is the functional domain of HAI-1 for inhibiting the HGF-converting activity of HGF activator. Furthermore, the presence of two Kunitz domains affected the inhibitory activity of HAI-1 against HGF activator, and it showed a similar, but not additive, level of inhibitory activity against trypsin when compared with that of the individual Kunitz domains. These results suggest that serine protease binding sites of Kunitz I and Kunitz II are located close to each other and that proteolytic processing to generate HAI-1 with only one Kunitz domain regulates the activity of HAI-1.

Hepatocyte growth factor activator inhibitor type 1 (HAI-1)\(^1\) is a Kunitz-type serine protease inhibitor (1) initially identified as a strong inhibitor of hepatocyte growth factor (HGF) activator. HGF activator is a blood coagulation factor XII-like serine protease that catalyzes proteolytic conversion of the inactive single-chain precursor of HGF to the active two-chain form in response to tissue injury (2, 3). The activated HGF is involved in cell growth during the repair of injured tissues (4–7). Furthermore, HAI-1 has been shown to be up-regulated along with repair of injured tissues (8, 9). Thus, HAI-1 may play a crucial role in tissue repair by regulating the activity of a growth factor-activating enzyme. HAI-1 was also identified from human breast milk in a complex with another serine protease, named matriptase (10). Matriptase is an epithelial cell-derived serine protease that has extracellular matrix-degrading activity and has been proposed to play a role in breast cancer invasion (11, 12). Thus, HAI-1 may also function as a regulator of tumor progression by controlling the activity of an extracellular matrix-degrading enzyme.

The primary translation product of HAI-1, which was predicted from the cDNA sequence, consists of characteristic structural domains, including two Kunitz domains, a low density lipoprotein receptor (LDLR)-like domain between the Kunitz domains, and a transmembrane domain (1). Immunoblotting analysis revealed that HAI-1 is first produced in a 66-kDa membrane-integrated form, and subsequent ectodomain shedding releases two major secreted forms from the cell surface into the extracellular space, and the sizes of the two secreted forms are 40/39 and 58 kDa (13). The 40/39- and 58-kDa HAI-1 have the same N-terminal sequence; thus, they are produced from the membrane-integrated form by different C-terminal processing. The 40/39-kDa HAI-1 has one Kunitz domain and shows strong inhibitory activity against the HGF-converting activity of HGF activator. The 58-kDa HAI-1 has two Kunitz domains and shows markedly weak inhibitory activity against HGF activator (13). Thus, the presence of the C-terminal region including the C-terminal Kunitz domain (Kunitz II) in the 58-kDa HAI-1 may interfere with the binding of HGF activator to the reactive site of the N-terminal Kunitz domain (Kunitz I), whereas elimination of this region by proteolytic processing could lead to strong binding of HGF activator to HAI-1.

The cell surface HAI-1 integrated in epithelial cell membranes has a high affinity for HGF activator and forms a complex with the serine protease. Treatment of the cells with phorbol 12-myristate 13-acetate or interleukin 1β markedly enhances release of the 58-kDa secreted form of HAI-1, but not the 40/39-kDa secreted form of HAI-1, from the cell surface (14). This regulated shedding appears to be mediated by a zinc-metalloprotease. Because the 58-kDa HAI-1 has a low affinity for HGF activator, this enzyme is released from the complex with HAI-1, and as a result, significant HGF activator activity is recovered in the conditioned medium (14). These observations suggest that the cell surface HAI-1 acts not only as an inhibitor but also as a specific acceptor of active HGF activator and that regulated ectodomain shedding ensures the concentrated pericellular activity of HGF activator under certain cellu-
lar conditions, such as tissue injury and inflammation.

Whereas regulated proteolytic processing of HAI-1 has been well characterized (13, 14), the roles of the characteristic structural domains in the inhibitory activity against serine proteases remain to be elucidated. Therefore, in this study, we constructed various HAI-1 mutant proteins and examined their inhibitory activity against HGF activator and trypsin. We found that Kunitz I had potent inhibitory activity against both HGF activator and trypsin, whereas Kunitz II had very weak inhibitory activity against HGF activator, although its potency against trypsin was equivalent to that of Kunitz I. These results indicate that Kunitz I is the functional domain of HAI-1 for inhibition of HGF activator. We also found that the presence of two Kunitz domains affected the inhibitory activity of HAI-1 against HGF activator and showed a similar, but not additive, level of the inhibitory activity against trypsin when compared with that of the individual Kunitz domains. These results suggest that serine protease binding sites of Kunitz I and Kunitz II are located close to each other and that proteolytic processing to generate HAI-1 with only one Kunitz domain regulates the activity of HAI-1.

EXPERIMENTAL PROCEDURES

Materials—Reagents were obtained as follows: CHAPS and bovine serum albumin (BSA) fraction V were obtained from Sigma, bovine pancreas trypsin was obtained from Roche Diagnostics GmbH, nonspecific mouse IgG was obtained from Organon Teknika Corp., Alexa 488-conjugated sheep IgG anti-mouse IgG was obtained from Molecular Probes, and enhanced chemiluminescence Western blotting detection reagents were obtained from Amersham Biosciences, Inc. Monoclonal antibodies (mAbs) against HAI-1, C76-18 and 1N7, and mAb against HGF activator, A23, were prepared as described previously (3, 8, 13). Single-chain HGF and HGF activator were prepared as described previously (2, 4).

Construction and Transfection of Expression Plasmids for HAI-1 Mutant cDNAs—We used the pME18S vector (15) to express various recombinant murine HAI-1 glycoproteins. The cDNA encoding full-length HAI-1 was prepared as described above for COS-7 cells, and then cultured in fresh serum-free medium. After 2–6 days, the conditioned medium for immunoblotting analysis was prepared as described above for COS-7 cells. To prepare cell extracts, the cells were washed with cold 0.9% NaCl and treated with 1 ml of 10% trichloroacetic acid for 15 min on ice. The trichloroacetic acid-insoluble fraction was pelleted by centrifugation. The supernatant was dissolved in 150 μl of 7 M urea containing 2% Triton X-100 and 5% 2-mercaptoethanol. After an additional centrifugation, the supernatant was recovered and analyzed for immunoblotting. The samples for immunoblotting were separated by SDS-PAGE under reducing conditions. The proteins were transferred to a nitrocellulose membrane (Hybond ECL; Amersham Biosciences, Inc.) and incubated with the anti-HAI-1 mAb for 2 h at 4 °C. The membrane was incubated with horseradish peroxidase conjugated secondary antibodies for 1 h. Immunoreactive proteins were visualized with an enhanced chemiluminescence Western blotting detection system.

Assay for Inhibitory Activity of HAI-1 Mutant Proteins against HGF Activator and Trypsin—Concentrated conditioned medium containing each HAI-1 mutant was mixed with 1.5 ng of HGF activator or 1.1 ng of trypsin in 40 μl of phosphate-buffered saline with 0.05% CHAPS and then incubated for 30 min at 37 °C. After the incubation, 5 μl of 1.5 mg/ml single-chain HGF (for HGF activator) or 6 μl of 1.0 mg/ml BSA (for trypsin) in phosphate-buffered saline containing CHAPS was added to the respective mixture, and the samples were incubated for an additional 2 h. The reaction products were then separated by SDS-PAGE under reducing conditions. The gel was stained with Coomassie Brilliant Blue, and the bands were scanned using a Flying- Spot Scanner CS-900 (Shimadzu). The inhibitory activity against HGF activator was estimated by calculating the ratio of the remaining single-chain HGF to two-chain HGF. The inhibitory activity against trypsin was estimated by calculating the remaining BSA.

Immunocytostaining—CHO cell clones expressing full-length HAI-1 and HAI-1-LK2M as well as MKN45 cells were cultured in 8-well glass chamber slides with eRDF medium containing 5% fetal bovine serum. At semiconfluence, the cells were washed with cold phosphate-buffered saline containing 0.1% gelatin and 0.1% NaN3 (washing buffer) and incubated with 200 μl of 50 μg/ml anti-HAI-1 mAb (C76-18) or 500 μg/ml control mouse IgG for 60 min on ice. After the incubation, the cells were washed with washing buffer and incubated with Alexa 488-conjugated anti-mouse IgG. The cells were then washed with washing buffer and visualized by a confocal immunofluorescence microscope. For detection of bound HGF activator, the cells were incubated with 5 μg/ml HGF activator for 30 min on ice. The cells were then washed with washing buffer and incubated with 200 μl of 50 μg/ml anti-HGF activator mAb (A23) before being visualized as described above.

RESULTS

Construction and Expression of HAI-1 Deletion Mutant cDNAs—The extracellular region of HAI-1 (Fig. 1) consists of a long N-terminal region (N), a Kunitz I (K1), an LDLR-like domain (L), and Kunitz II (K2). To determine the role of each domain within the extracellular region of HAI-1 in the inhibitory activity against serine proteases, we constructed five deletion mutant cDNAs (Fig. 1). To obtain sufficient amounts of secreted proteins in conditioned medium, we deleted the transmembrane region in all constructs. HAI-1-NK1LK2 contained almost the entire extracellular region including Kunitz I and Kunitz II. HAI-1-NK1 contained Kunitz I but lacked the LDL-like domain and Kunitz II, whereas HAI-1-NKL1 contained only Kunitz II. HAI-1-LK2 contained Kunitz II but lacked the N-terminal region and Kunitz I. HAI-1-N consisted of only the N-terminal region. The constructs were inserted into expression vector pME18S and transiently transfected into COS-7 cells. Proteins secreted into the conditioned medium were analyzed by immunoblotting. Two monoclonal antibodies, C76-18 and 1N7, were used for the immunoblotting. C76-18, which was directed against an epitope located in the N-terminal region, recognized HAI-1-NK1LK2, HAI-1-NK1, HAI-1-NKL1 and HAI-1-N, whereas 1N7, which was directed against an epitope located in Kunitz II, recognized HAI-1-NK1LK2 and HAI-1-LK2. The immunoblotting analysis revealed that sufficient amounts of HAI-1 mutant proteins were secreted into the conditioned medium (Fig. 2). It has been shown that MKN45
Characterization of Kunitz Domains in HAI-1

human gastric carcinoma cells produce two major secreted forms of HAI-1, 40/39- and 58-kDa HAI-1 (13). However, the 40/39-kDa form was barely detected in the conditioned medium of COS-7 cells transfected with full-length HAI-1 or HAI-1-NK1LK2 (Fig. 2A, lanes 3 and 4), indicating that its contribution to the inhibitory activity was negligible. Thus, the inhibitory activity present in the conditioned medium depended mostly on HAI-1 proteins with two Kunitz domains. The mutant proteins were quantified by scanning densitometry of the immunoblots using purified HAI-1 as a standard and assayed for their inhibitory activity against serine proteases.

Assay for the Inhibitory Activity of HAI-1 Mutant Proteins against HGF Activator and Trypsin—We examined the inhibitory activity of the HAI-1 mutant proteins against two serine proteases, HGF activator and trypsin. To assess the inhibitory activity against HGF activator, the protease was mixed with each mutant protein and incubated for 30 min to allow formation of the enzyme-inhibitor complex. Next, single-chain HGF was added as a substrate, and the mixture was incubated for an additional 2 h. The samples were then analyzed by SDS-PAGE. To assess the inhibitory activity against trypsin, the enzyme was mixed with each mutant protein and incubated for 30 min. Next, BSA was added as a substrate, and the mixture was incubated for an additional 2 h before being analyzed by SDS-PAGE. Fig. 3 shows representative patterns of the SDS-PAGE analysis. Single-chain HGF was converted to two chains (a heavy chain and a light chain) by HGF activator in the absence of HAI-1 (Fig. 3A, lanes 2 and 4). This conversion was inhibited in the presence of the conditioned medium of MKN45 cells (Fig. 3A, lane 3), wild-type HAI-1 (Fig. 3A, lane 5), or mutant proteins that contained Kunitz I (Fig. 3A, lane 6–8), but not in the presence of mutant proteins that contained only the N-terminal region (Fig. 3A, lane 9) or Kunitz II (Fig. 3A, lane 10). These results suggest that Kunitz I, and not Kunitz II, is the functional domain for inhibition of the HGF-converting activity of HGF activator. BSA was degraded by trypsin in the absence of HAI-1 (Fig. 3B, lanes 2 and 4). This degradation was inhibited in the presence of the conditioned medium of MKN45 cells (Fig. 3A, lane 3), wild-type HAI-1 (Fig. 3B, lane 5), or mutant proteins that contained Kunitz I and/or Kunitz II (Fig. 3B, lanes 6–8 and 10), but not in the presence of a mutant protein that consisted of only the N-terminal region (Fig. 3B, lane 9). These results indicate that both Kunitz I and Kunitz II have inhibitory activity against trypsin.

Dose Dependence of the Inhibitory Activity of HAI-1 Mutant Proteins against HGF Activator and Trypsin—To quantitate the inhibitory activity of the Kunitz domains in HAI-1, the dose dependence of the inhibitory activity of mutant proteins was examined. Fig. 4A shows the dose-dependence curves for the inhibitory activity of deletion mutants against HGF activator. HAI-1-NK1 showed a dose-dependence curve similar to that of HAI-1-NK1L, and the concentration for 50% inhibition was about 2 nM. HAI-1-NK1LK2 showed a dose similar to that of purified 58-kDa HAI-1, and the concentration for 50% inhibition was about 6 nM. The concentration of HAI-1-LK2 for 50% inhibition was about 19 nM. These results indicate that HAI-1 with only Kunitz I shows the most potent inhibitory activity against HGF activator and that HAI-1 with two Kunitz domains is about 3-fold less potent, whereas HAI-1 with only Kunitz II is about 10-fold less potent. Fig. 4B shows the dose-dependence curves of the inhibitory activity against trypsin. All HAI-1 mutant proteins showed similar dose-dependence curves, and the concentration for 50% inhibition was about 2 nM.

Immunocytochemical Analysis for the Binding of a Membrane-integrated Form of HAI-1 with Only Kunitz II to HGF Activator—The membrane-integrated form of full-length HAI-1 showed strong binding to HGF activator, whereas the secreted form of HAI-1 containing almost the entire extracellular region (the 58-kDa HAI-1) showed rather weak binding (14). Thus, it is possible that the membrane-integrated form of HAI-1-LK2
may be capable of binding to HGF activator. To examine this possibility, cDNA encoding the HAI-1-LK2M protein that contained the transmembrane region and C-terminal cytoplasmic tail of HAI-1 (Fig. 5A) was constructed and stably transfected into CHO cells. The ability of the expressed proteins to bind HGF activator was tested by immunocytostaining. For positive controls, CHO cells expressing full-length HAI-1 and MKN45 cells expressing a high level of endogenous HAI-1 were also analyzed. Immunoblotting analysis of the cell extracts suggested that the membrane-integrated form of HAI-1-LK2M was expressed in the transfected CHO cells (Fig. 5B, lane 6). Immunocytostaining with a mAb against HAI-1 confirmed the presence of HAI-1-LK2M on the cell surface (Fig. 5C, g). However, no binding of HGF activator was observed on the surface of the cells expressing HAI-1-LK2M (Fig. 5C, k). On the other hand, obvious binding of HGF activator was observed on the cell surface of the positive control cells (Fig. 5C, j and l). These results indicate that Kunitz II of HAI-1 has a low affinity for HGF activator both in the secreted form and in the membrane-integrated form.

Analysis of the Inhibitory Activity of HAI-1 Mutant Proteins with a Point Mutation in the P1 Positions—The amino acid residues in the P1 positions of the reactive sites of various Kunitz domains were assigned as essential for their inhibitory activity (18, 19). To further characterize the roles of the Kunitz domains in the inhibitory activity of HAI-1-NK1LK2, we constructed cDNAs encoding HAI-1-NK1LK2 mutants in which an arginine residue (Arg260) of the P1 position in Kunitz I or a lysine residue (Lys385) of the P1 position in Kunitz II was individually changed to a leucine residue (R260L and K385L, respectively). In addition, cDNA encoding a HAI-1-NK1LK2 mutant in which both Arg260 and Lys385 were changed to leucine was also constructed (R260L/K385L). These cDNAs as well as cDNA encoding HAI-1-NK1LK2 were stably transfected into CHO cells because transient transfection of these mutant cDNAs into COS-7 cells did not produce sufficient amounts of mutant proteins. Cell lines that stably produced and secreted proteins of each mutant were obtained. Immunoblotting analysis showed that sufficient mutant proteins with molecular mass of 58 kDa were obtained in the conditioned medium (Fig. 6). These proteins were assayed for their inhibitory activity against HGF activator and trypsin.

Fig. 7A shows the dose-dependence curves of the inhibitory activity against HGF activator. HAI-1-NK1LK2 with a mutation in Kunitz II (K385L) showed significantly weaker inhibitory activity than intact HAI-1-NK1LK2, and the concentration for 50% inhibition was 13 nM. A mutation in Kunitz I (R260L) led to a further reduction in the inhibitory activity, and the
concentration for 50% inhibition was 42 nM. Almost no activity was observed in the protein with point mutations in both Kunitz domains (R260L/K385L). Fig. 7B shows the dose-dependence curves of the inhibitory activity against trypsin. A mutation in Kunitz I (R260L) or Kunitz II (K385L) did not significantly affect the inhibitory activity. Almost no activity was observed in the protein with point mutations in both Kunitz domains (R260L/K385L).

**DISCUSSION**

In this study, we found that the N-terminal Kunitz domain (Kunitz I) of HAI-1 had strong inhibitory activity against HGF activator, whereas in contrast, the C-terminal Kunitz domain (Kunitz II) of HAI-1 had only weak inhibitory activity. From these findings, we concluded that Kunitz I is the functional domain of HAI-1 that is responsible for inhibition of the HGF-converting activity of HGF activator. Ectodomain shedding of the membrane-integrated HAI-1 produces the 40/39- and 58-kDa secreted forms of HAI-1. The 40/39-kDa HAI-1 with only Kunitz I shows much stronger inhibitory activity against HGF activator than the 58-kDa HAI-1 with Kunitz I and Kunitz II (13). Moreover, activated matriptase forms a tight complex with HAI-1, and the molecular mass of HAI-1 isolated from the complex was determined to be 40 kDa (20), suggesting that HAI-1 with only Kunitz I has strong inhibitory activity against matriptase, although functional analysis of Kunitz II against matriptase has not yet been performed. Thus, proteolytic processing to generate HAI-1 with only Kunitz I (the 40/39-kDa HAI-1) appears to be a crucial step to produce a form of HAI-1 that has strong affinity for both HGF activator and matriptase.
An LDLR-like domain was assigned between Kunitz I and Kunitz II of HAI-1 (1). It has been suggested that this domain may be involved in formation of the inhibitor-enzyme complex because it contains many negatively charged amino acid residues (1). In the present study, however, we showed that the absence of this domain did not affect the inhibitory activity of Kunitz I against both HGF activator and trypsin under our assay conditions. Thus, the LDLR-like domain may not be involved in association of the inhibitor with proteases. It is possible that this domain regulates the proteolytic processing of HAI-1, as was proposed previously (13).

Although the individual Kunitz domains of HAI-1 had similar inhibitory activity against trypsin, the HAI-1 protein with two Kunitz domains also showed a similar, but not additive, level of inhibitory activity compared with that of the individual Kunitz domains. Moreover, a mutation in the P1 position of Kunitz II of HAI-1-NK1LK2 did not significantly affect the inhibitory activity against trypsin. These results suggest that the serine protease binding sites of Kunitz I and Kunitz II are located close to each other, and thus only one molecule of trypsin can associate with HAI-1 even if it has two Kunitz domains. The HAI-1 protein with two Kunitz domains showed much weaker inhibitory activity against HGF activator than the HAI-1 protein with only Kunitz I. Thus, the closely located Kunitz II appears to interfere with the binding of Kunitz I to HGF activator. A substitution of the lysine residue at the P1 position of Kunitz II with a leucine residue reduced the inhibitory activity of HAI-1-NK1LK2 against HGF activator but not against trypsin, suggesting that a small structural change around the P1 position affects the association of Kunitz I with HGF activator but not with trypsin. The molecular mass of HGF activator is 34 kDa (1), whereas that of trypsin is only 23 kDa. This difference in molecular mass may explain why trypsin has easier access than HGF activator to the HAI-1 protein with two Kunitz domains. The high-resolution crystallographic studies of bikunin, a serine protease inhibitor with two Kunitz domains, revealed an interrelation of positions of the domains (23). The two Kunitz domains of bikunin are packed close together, but the protease binding site of the first domain is unobstructed by the second. In contrast, the protease binding site of the second domain is close to the first, and thus protease binding is affected by the presence of the first domain (23, 24). In the case of HAI-1, the protease binding of each Kunitz domain was affected by the presence of other domain, suggesting that the three-dimensional structure of HAI-1 with two Kunitz domains is different from that of bikunin. The presence of the LDLR-like domain between the two Kunitz domains of HAI-1 may contribute to a three-dimensional structure different from that of bikunin because no amino acid residue is present in the linking region between the two Kunitz domains of bikunin (23). It should be noted that the membrane-integrated form of full-length HAI-1 has a high affinity for HGF activator, whereas the secreted 58-kDa form of HAI-1 has a weak affinity for HGF activator (14). This observation suggests that Kunitz II in the membrane-integrated HAI-1 does not interfere with the binding of Kunitz I to HGF activator and that proteolytic processing to generate the secreted form of HAI-1 brings Kunitz I and Kunitz II closer together and thus causes Kunitz II to interfere with the binding of Kunitz I to HGF activator.

Because the proteolytic processing to generate the 40/39-kDa HAI-1 occurs between Kunitz I and Kunitz II (13), there is a possibility that the processing may also generate a HAI-1 protein with only Kunitz II in a secreted and/or membrane-integrated form, although no such a molecule of HAI-1 has yet been identified. A serine protease inhibitor named trypstatin, which

**Fig. 7.** Dose dependence of the inhibitory activity of HAI-1 point mutation proteins against HGF activator and trypsin. Inhibitory activity against HGF activator (A) and trypsin (B) was assayed as described in the Fig. 4 legend.

Although Kunitz II showed weak activity against HGF activator, it had strong inhibitory activity against trypsin, equivalent to that of Kunitz I. Thus, Kunitz II has the potential to function as a serine protease inhibitor but has only a weak association with HGF activator. The amino acid residues in the P1 positions of the reactive sites in various Kunitz domains are essential for their inhibitory activity (18, 19). The amino acid residue of the P1 position in Kunitz I is an arginine, and that in Kunitz II is a lysine. HGF activator cleaves the single-chain HGF precursor after the arginine residue of the P1 position (2, 21), suggesting that HGF activator has the ability to bind an arginine residue at the P1 position, and thus it has strong affinity for Kunitz I, but only weak affinity for Kunitz II. However, some Kunitz-type serine protease inhibitors with an arginine residue at the P1 position did not inhibit the HGF-converting activity of HGF activator (22). Several amino acid residues around the P1 positions differ between Kunitz I of HAI-1 and the Kunitz domains of these inhibitors (1). Thus, in addition to the arginine residue of the P1 position, some of the different residues in Kunitz I may also contribute to the strong affinity for HGF activator. Because trypsin cleaves substrate proteins after the arginine and lysine residues of their P1 positions (18), it can associate with both Kunitz I and Kunitz II. Furthermore, the difference in amino acid residues around the P1 positions may not affect the binding of trypsin to Kunitz I and Kunitz II.
consists of only the second Kunitz domain of bikunin, was identified from mast cell (25, 26). It inhibits factor Xa, tryptase, trypsin, and chymase. Thus, the proteolytic processing of bikunin to eliminate the first Kunitz domain leads to an activation of the second Kunitz domain. Kunitz II of HAI-1 has strong inhibitory activity against trypsin, even in the presence of Kunitz I. However, similar to the second Kunitz domain of bikunin, it may also have inhibitory activity against other serine proteases when Kunitz I is eliminated by proteolytic processing. Thus, identification of a HAI-1 protein with only Kunitz II and its cognate serine proteases will reveal the novel biological functions of Kunitz II.

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Functional Characterization of Kunitz Domains in Hepatocyte Growth Factor Activator Inhibitor Type 1
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