The ability of plasma proteinase inhibitors to inactivate human chymase, a chymotrypsin-like proteinase stored within mast cell secretory granules, was investigated. Incubation with plasma resulted in over 80% inhibition of chymase hydrolytic activity for small substrates, suggesting that inhibitors other than α2-macroglobulin were primarily responsible for chymase inactivation. Depletion of specific inhibitors from plasma by immunoadsorption using antisera against individual inhibitors established that α1-antichymotrypsin (α1-AC) and α1-proteinase inhibitor (α1-PI) were responsible for the inactivation. Characterization of the reaction between chymase and each inhibitor demonstrated in both cases the presence of two concurrent reactions proceeding at fixed relative rates. One reaction, which led to inhibitor inactivation, was about 3.5 and 4.0-fold faster than the other, which led to chymase inactivation. This was demonstrated in linear titrations of proteinase activity which exhibited endpoint stoichiometries of 4.5 (α1-AC) and 5.0 (α1-PI) instead of unity, and SDS gels of reaction products which exhibited a banding pattern indicative of both an SDS-stable proteinase-inhibitor complex and two lower Mₙ inhibitor degradation products which appear to have formed by hydrolysis within the reactive loop of each inhibitor. At inhibitor concentrations approaching those in plasma where inhibitor to chymase concentration ratios were in far excess of 4.5 and 5.0, the rate of chymase inactivation by both serpin inhibitors appeared to follow pseudo-first order kinetics. The "apparent" second order rate constants of inactivation determined from these data were about 3000-fold lower than the rate constants reported for human neutrophil cathepsin G and elastase with α1-AC and α1-PI, respectively. This suggests that chymase would be inhibited about 650-fold more slowly than these proteinases when released into plasma. These studies demonstrate that although chymase is inactivated by serpin inhibitors of plasma, both inhibitors are better substrates for the proteinase than they are inhibitors. This finding along with the slow rates of inactivation indicates that regulation of human chymase activity may not be a primary function of plasma.

Mast cells from human (1, 2), dog (3, 4), and rat (5-7) contain substantial amounts of serine proteinases with chymotrypsin-like specificity (8). These proteinases, termed chymases, are constituents of cytoplasmic secretory granules (9-11) where they appear to be packaged in an active form (12-14) bound to heparin (5, 9, 10, 14, 15). They are released concomitant with histamine when mast cells degranulate in vitro (14, 16, 17). The localization of the chymases in secretory granules and their release coincident with degranulation suggests chymases function extracellularly. The biological role of these enzymes has not been established with certainty.

Many serine proteinases which function in plasma or tissue during inflammation appear to be regulated by protein-proteinase inhibitors found in plasma (18, 19). About 10% of the proteins in plasma are proteinase inhibitors (18). Most numerous in variety are those of the serpin superfamily which specifically inhibit serine class proteinases (18, 19). Also found are the general proteinase inhibitor α2M and the Kunitz type inhibitor inter-α-trypsin inhibitor (18). Several of these inhibitors react with chymotrypsin-like proteinases. The high rate constant for the inactivation of cathepsin G (kₐ = 5.1 × 10⁷ M⁻¹ s⁻¹), the major chymotrypsin-like proteinase of human neutrophils, by the serpin α1-AC, suggests that this inhibitor is responsible for regulating the activity of cathepsin G in vivo (20). In a similar fashion neutrophil elastase is believed to be regulated by the serpin α1-PI (20). Lack of functional α1-PI has been correlated with production of lung tissue deterioration in emphysema (18, 20).

Although the mature mast cell is usually found as a resident of tissue such as the dermis of skin (21), evidence obtained primarily from rodents indicates that mast cells are produced from stem cells in the bone marrow (22); therefore, like neutrophils, they may be considered part of the hematolymphoid system involved in host defense. This suggests that the mechanisms regulating neutrophil serine proteinases may also apply to mast cell serine proteinases, despite the location of these cells in tissues. When mast cell degranulate during the allergic response, for example, histamine is released from secretory granules. The action of histamine produces local increased vascular permeability (23) which may lead to an influx of plasma proteins, including inhibitors, into tissues. These inhibitors could then regulate chymase activity. The purpose of the present study was to determine whether human plasma proteinase inhibitors are effective inhibitors of human chymase, which is presumably also secreted from mast cells when they degranulate.

The abbreviations are: α2M, α2-macroglobulin; α1-AC, α1-antichymotrypsin; α1-PI, α1-proteinase inhibitor; PVDF, polyvinylidene difluoride; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; NA, p-nitroaniline; SDS, sodium dodecyl sulfate.
**EXPERIMENTAL PROCEDURES**

**Materials**—Proteinase inhibitors α1-PI and chymostatin were from Calbiochem. αM, PMSF, active site titrants, bovine pancreatic proteinases, and peptide-NA substrates were from Sigma. Antisera against α1-PI (M-1895), α2-AC (DAKO A-012), and α1-PI (DAKO A-012) were obtained from commercial sources. Standard proteins for gel analysis were from Bio-Rad, except that B chain of insulin (M, = 3000) and aprotinin (M, = 6500) were from Sigma. Polyvinylidene difluoride (PVDF) membranes were from Millipore.

**Polyvinylidene difluoride (PVDF) membranes**—Human blood was collected by venipuncture into containers having EDTA (7.5 mg/5 ml blood).

**Determination of Inhibitor and Proteinase Concentrations**—For titrations of chymase with purified α1-PI or chymostatin (31), all measurements were made spectrophotometrically. Proteinase activity was measured as described previously (29, 31). The reaction of chymase with α1-PI was measured under pseudo first order conditions, where E is the residual activity of proteinase, E0 is initial activity, and t is time of incubation. The reaction of chymase with α1-PI was measured under pseudo first order conditions, where E is the residual activity of proteinase, E0 is initial activity, and t is time of incubation.

**Human Chymase with Plasma Proteinase Inhibitors**—Titration with chymase and plasma proteinase inhibitors was performed similarly as described for chymotrypsin except that data were collected over longer periods of incubation. In this method, it was found (3, 4) that the reaction of chymase with plasma inhibitors was biphasic, with the first phase representing the inhibition of chymase by high concentrations of plasma inhibitors and the second phase representing the inhibition of chymase by low concentrations of plasma inhibitors. The second phase of the reaction was used for the determination of the inhibition constant of plasma inhibitors.

**Titration of Chymase with Purified Serpin Inhibitors**—The reaction of chymase with α1-PI was measured under pseudo first order conditions, where E is the residual activity of proteinase, E0 is initial activity, and t is time of incubation. The reaction of chymase with plasma inhibitors was biphasic, with the first phase representing the inhibition of chymase by high concentrations of plasma inhibitors and the second phase representing the inhibition of chymase by low concentrations of plasma inhibitors. The second phase of the reaction was used for the determination of the inhibition constant of plasma inhibitors.

**Purification of Proteinases and Inhibitors**—Bovine pancreatic chymotrypsin was further purified on phenylbutyrylamino-CH-Sepharose. Elution was achieved by treatment with 0.5 M NaCl. Except for titrations of chymotrypsin with aprotinin, all titrations were measured spectrophotometrically in a Beckman DU 8 spectrophotometer.

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were the primary inhibitors of chymase in plasma. Inhibitory adsorb out inhibitors from plasma as described under "Experimental Procedures." Plasma was consecutively depleted of various dilutions of plasma. Assays were performed in 100 µl containing substrate, and then immediately assayed for hydrolytic activity.

Identification of Inhibitors—The inhibitory activity of plasma to inhibit chymase, demonstrating that they are responsible for the inactivation of chymase, IgGs from antisera to the relationship of chymase and chymotrypsin were incubated for 10 min (25 °C) in plasma diluted in 0.45 M Tris-HCl (pH 8.0), 1.0 M NaCl at 25 °C as described under "Experimental Procedures." Chymase concentration was 250 nM. Samples were removed and assayed at indicated times to determine the effect of incubation period on the inactivation reaction.

Our studies show that whole plasma is a more efficient inhibitor of chymotrypsin than of chymase. Part of the reason for this is that the range of inhibitors against chymase, primarily α1-PI, and α1-AC, was narrower than for chymotrypsin. Another reason, which will be subsequently described, is that both α1-PI and α1-AC are not highly efficient inhibitors of chymase. Inhibitory activity toward chymotrypsin still remained, however. Chymotrypsin has been shown to react with several other plasma inhibitors including α2-antiplasmin, heparin cofactor II and α1-antitrypsin inhibitor (18).

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unity was determined (Fig. 3, filled symbols). The stoichiometry of reaction remained constant over a 14-fold variation in proteinase concentrations (92 nM to 1.3 \( \mu \) M), strongly indicating an irreversible mechanism of inactivation (Fig. 3).

The high stoichiometry of reactions was not due to the presence of contaminating protein or denatured inhibitor because initial \( \alpha_1 \)-AC concentrations were based on titrations obtained at other proteinase concentrations. Reactions represented by filled symbols and those at pH 6.0 in 0.05 M Na\( \text{H} \)P (pH 6 or 6), 0.6 M NaCl and reactions were performed at 25 \(^\circ\)C. Controls which contained chymase but no inhibitor are represented by open boxes (pH 6.9) and closed boxes (pH 8.0). Inset is a semi-log plot data obtained at pH 6.0. Reactions were performed in 0.5 ml and at various times 0.1-ml samples were removed and diluted 10-fold with normal assay buffer containing substrate. Fractional activity (FA) was determined as described in Fig. 1.

Fig. 4. Kinetic analyses of human chymase inactivation with \( \alpha_1 \)-AC at pH 8.0 (solid circles) and pH 6.0 (open circles). I/E was 4.5 and concentration of chymase was 29 nM. Buffers were 0.05 M Na\( \text{H} \)P (pH 8 or 6), 0.6 M NaCl and reactions were performed at 25 \(^\circ\)C. Controls which contained chymase but no inhibitor are represented by open boxes (pH 6.9) and closed boxes (pH 8.0). Inset is a semi-log plot data obtained at pH 6.0. Reactions were performed in 0.5 ml and at various times 0.1-ml samples were removed and diluted 10-fold with normal assay buffer containing substrate. Fractional activity (FA) was determined as described in Fig. 1.

For further evidence on the hydrolysis of \( \alpha_1 \)-AC by chymase, it was obtained by identification of a second cleavage product of much lower apparent \( M_r \) (about 4000) on a more highly cross-linked gel (Fig. 5, lane 11). Production of this fragment by hydrolysis from either terminal region of \( \alpha_1 \)-AC would help to account for the production of the 52-kDa product. The origin of the low \( M_r \) peptide as a COOH-terminal fragment was indicated by elucidation of its NH\( _2 \)-terminal sequence (20 cycles). Sequence analysis was performed on peptide electroblotted from the gel onto PVDF membranes as described under “Experimental Procedures.” Two NH\( _2 \)-terminal sequences were found corresponding to hydrolysis between residues Leu\( ^{50} \)Ser\( ^{50} \), the P1-P1' site of the inhibitor, and Leu\( ^{341} \)-Val\( ^{342} \) (P2-P2'). Based on recoveries of phenylthiohydantoin derivatives, both peptides were present in a 2:1 ratio. The

\( ^3 \) Sequences of inhibitors and nomenclature described in Ref. 19.
The rate of inhibitor degradation was 3.5-fold faster than proteinase inactivation and inhibitor degradation. The lower product (47,000) than native inhibitor showed the formation of a higher product (62,000) obtained in the presence of 100 μg/ml chymostatin is shown in lane 7. All reactions containing chymase were performed at pH 8.0 in 0.1 M Tris-HCl, 0.8-1.0 M NaCl, except for reactions analyzed in lanes 9 and 10 which were performed at pH 6.0 in 0.05 M NaP, 0.8 M NaCl. Products analyzed in lanes 1-6 were denatured in SDS without heat or reducing agent as described under “Experimental Procedures,” whereas in lanes 7-12, PMSF (2 mM for 15 min) was added at the conclusion of the incubation to ensure that any possible remaining proteinase activity was inhibited prior to denaturation in SDS. Addition of reducing agent or heating for 2 or 10 min (lanes 7-12) had no effect on the banding pattern of products produced by chymase. Except for lane 12 where 2.3 μg of PMSF-inhibited proteinase was resolved, chymase (±0.6 μg/lane) was difficult to detect in controls. Chymase migrates on SDS gels as a diffuse band at M, 30,000 (1, 4, 30). The diffuse banding pattern results from glycosylation (N. M. Schechter, unpublished observation). Samples corresponding to 1.6, 3.8, and 48 μg of α1-AC were analyzed on lanes 1-6, 7-10, and 11 and 12, respectively.

The similarity in the titration data over a range of different proteinase concentrations indicates that the rates of reaction over these pathways are linked in a fixed ratio which is independent of inhibitor and proteinase concentrations. The rate of inhibitor degradation was 3.5-fold faster than proteinase inactivation. Thus at pH 8.0, α1-AC is a better substrate for chymase than it is an inhibitor.

**Reaction of Chymase with α1-PI**—Similar studies were performed to characterize the reaction of chymase with α1-PI. Titration of chymase with α1-PI exhibited a linear relationship having an endpoint of 5.0 instead of unity (Fig. 6, solid symbols). This suggests that α1-PI, like α1-AC, is both a substrate and an inhibitor of chymase and that these reactions may occur by a two-pathway mechanism as observed for α1-AC. SDS-gel analysis of the reaction products on 10% gels (Fig. 7, lanes 5 and 8) showed the formation of a higher (62,000) and a lower M, product (47,000) than native inhibitor (52,000). Again, these products are indicative of proteinase inactivation and inhibitor degradation. The lower M, product was not observed in similar studies with chymotrypsin (Fig. 7, lanes 2 and 3). The higher M, product (62,000) obtained after the reaction with chymase and chymotrypsin had a significantly lower M, than expected for a complex between α1-PI and either proteinase. The reason for this is unclear. However, formation of all reaction products was inhibited by PMSF and chymostatin (Fig. 7, lanes 4, 6, and 7) indicating that active chymase was required to form both products.

The reaction of α1-PI with chymase was also dependent on pH and ionic strength. However, with this inhibitor, lowering the pH had the opposite effect of that observed with α1-AC; i.e. more inhibitor degradation relative to proteinase inhibition was observed at pH 6.0 (Fig. 6, open circles) than at pH 8.0. This change in activity did not result from denaturation of α1-PI at the lower pH, as demonstrated in controls (not shown). The effect of ionic strength was similar to that
observed with α₁-AC (Fig. 6, inset).

SDS gels of reaction products produced at pH 6.0 are shown in Fig. 7 (lane 10). The gel was highly cross-linked and shows the presence of a small peptide with a $M_r$ of about 4000. After electrophoretic transfer of the protein onto PVDF membranes, NH$_2$-terminal sequence analysis was performed on the small peptide. Two NH$_2$-terminal sequences were obtained indicating that these peptides were produced by hydrolysis at Phe$^	ext{15}$-Leu$^	ext{35}$ (P7-P6) and Met$^	ext{31}$-Ser$^	ext{32}$, the PI-P$_i$ site of the inhibitor. In contrast to that observed with α₁-AC, recoveries of phenylalanyl-P$_i$-Ala$^	ext{1}$ were noted at about 1 μM, and not the PI-P$_i$ site predominated. It was found in a 15:1 ratio over the second hydrolytic site. These data also indicate that α₁-PI is a better substrate for chymase than it is an inhibitor.

**Estimated Rates of Chymase Inactivation in Plasma** — The rate of chymase inactivation by α₁-AC and α₁-PI at $I/E$ values substantially greater than those required for complete inactivation (i.e., $>4.5$ and 5.0, respectively) appeared to be pseudo first order over a range of inhibitor concentrations (Fig. 8). The highest concentration of inhibitor used, about 1 μM, is approaching the values of these inhibitors in plasma (6.4 μM for α₁-AC and 52 μM for α₁-PI as reported in Ref. 34). The

**TABLE II**

| Inhibitor | Human chymase | Chymotrypsin | Cathepsin G |
|-----------|---------------|--------------|-------------|
| Neutral pH |               |              |             |
| α₁-AC     | $k_u$ (μM$^{-1}$ s$^{-1}$) | $2.1 \times 10^4$ | $3.8 \times 10^6$ | $5.1 \times 10^7$ |
|           | $t_{1/2}$ (μM) | 5.4 s       | 0.002 s     |             |
| α₁-PI     | $k_u$ (μM$^{-1}$ s$^{-1}$) | $7.5 \times 10^6$ | $2.4 \times 10^6$ | $4.1 \times 10^6$ |
|           | $t_{1/2}$ (μM) | 1.8 s       | 0.032 s     |             |
| pH 6.0    |               |              |             |
| α₁-AC     | $k_u$ (μM$^{-1}$ s$^{-1}$) | $1.6 \times 10^4$ | $1.0 \times 10^5$ |
| α₁-PI     | $k_u$ (μM$^{-1}$ s$^{-1}$) |             |             |

*a $k_u$ reported are apparent values obtained from slopes of regression lines in Fig. 8. k$_u$ at pH 6.0 were determined under pseudo first order conditions using $I/E$ values of 9.4 and 8.2 (average difference ± 0.15 × 10$^6$, n = 2).

*b $k_u$ with α₁-AC at neutral pH was determined in PBS and value reported was the average of three experiments; S.D. = ± 0.2 × 10$^6$. k$_u$ with α₁-PI was determined in PBS. k$_u$ for the reaction with α₁-AC at pH 6.0 was determined in 0.01 M NaP (pH 6.0), 0.2 M NaCl. Beatty et al. (20) reported $k_u$ of 6.0 × 10$^6$ M$^{-1}$ s$^{-1}$ and 5.9 × 10$^6$ M$^{-1}$ s$^{-1}$ for α₁-AC and α₁-PI, respectively, at pH 8.0.

“Apparent” rate constants of inactivation obtained from the slopes in Fig. 8 are reported in Table II. Using these values and the concentration of each inhibitor in plasma, the halftime of inactivation for chymase by each inhibitor was estimated (Table II). Because the concentration of α₁-PI is about 10-fold higher than α₁-AC, it would appear to be 3-fold better physiological inhibitor. Based on the combined concentrations of both inhibitors, the $t_{1/2}$ for chymase in plasma would be 1.3 s. This is about 650-fold slower than estimated for neutrophil cathepsin G, which reacts most rapidly with α₁-AC. The time of inactivation of chymase may even be somewhat slower than this because these studies were performed in 2.0 M NaCl and for both inhibitors the rate of inhibitor hydrolysis to proteinase inactivation increased as the ionic strength was lowered.
The second-order rate constant was also determined for the reaction of chymase with $\alpha_1$-AC at pH 6.0, where inhibitor degradation was barely detectable (Table II). This value was lower than observed for chymotrypsin at this pH, suggesting that even with the degradation pathway nearly eliminated, $\alpha_1$-AC is still not an efficient inhibitor of chymase.

**DISCUSSION**

Even though mature mast cells are found almost exclusively in tissue such as the dermis of skin, current evidence indicates that these cells originate from precursor cells within the bone marrow. As a cell of hemopoietic origin, it might be consistent that serine proteinases secreted from mature mast cells during degranulation are regulated by plasma proteinase inhibitors in the same fashion as human neutrophil cathespin G and elastase. The release of histamine and other vasoactive components from mast cells during degranulation may produce a rapid influx of plasma components into tissue so that released proteinases would be quickly inhibited before they can diffuse from the site of secretion.

Our study demonstrates that plasma components irreversibly inhibit human chymase, a chymotrypsin-like protease found within mast cell secretory granules. The serpins $\alpha_1$-AC and $\alpha_1$-PI were identified as the primary inhibitors and both were shown to react with chymase forming products characteristic of the SDS-stable proteinase-inhibitor endproducts associated with serpin inhibition. The titration data with whole plasma also suggest that $a_2M$ may inhibit chymase as well. However, only 20% of the inactivation could be attributed to $a_2M$, indicating it is not the primary inhibitor of chymase in whole plasma. Removal of the above three inhibitors from plasma virtually eliminated activity against chymase but did not totally eliminate activity against chymotrypsin. This indicates a more limited variety of inhibitors in plasma for chymase than might be expected for a chymotrypsin-like protease.

Studies on the interaction of chymase with the individual serpin inhibitors demonstrated that the reaction of this protease with $\alpha_1$-AC and $\alpha_1$-PI was not as highly efficient as reported for neutrophil serine proteinases. Part of the reason for the inefficiency was that both inhibitors were good substrates for chymase. Under conditions approaching physiological ionic strength and pH, the rate of $\alpha_1$-AC and $\alpha_1$-PI degradation by chymase was 3.5 and 4.0-fold faster than its rate of inhibition, thus requiring a 4.5 and 5.0 stoichiometric ratio of each serpin to inactivate chymase. Actual rates of chymase inhibition for concentrations of serpins present in plasma were estimated from apparent second-order rate constants determined at excessive I/E ratios where the concentration of inhibitor was approaching that observed in normal plasma. The $k_{in}$ inactivation of chymase in plasma was 5.4 and 1.8 s $^{-1}$ with $\alpha_1$-AC and $\alpha_1$-PI, respectively. These values indicate chymase would be inhibited 650-fold more slowly than cathespin G if released into plasma (20). These values are also slower than those calculated for chymotrypsin, a pancreatic protease that is not found in plasma.

At pH 6.0, the degradation of $\alpha_1$-AC by chymase was reduced so that the $k_d$ of the inhibitor with the proteinase could be measured in the nearly complete absence of the degradation pathway. A $k_d$ of $1.6 \times 10^4$ M$^{-1}$ s$^{-1}$ was determined. This value was about 6-fold lower than observed for chymotrypsin at the same pH, suggesting that the interaction of chymase with $\alpha_1$-AC is slow even in the absence of the inhibitor degradation reaction. In the case of $\alpha_1$-PI, lowering the pH increased the rate of inhibitor hydrolysis relative to proteinase inactivation. A stoichiometry of 9 was required to fully inactivate chymase. These observations at low pH along with those at higher pH indicate that plasma would not efficiently inhibit chymase within the pH range of 6-8.

The slow apparent rate constants of inactivation suggest chymase may have sufficient time to inactivate both inhibitors through multiple nonspecific cleavages. However, the linearity and constancy of the titration curves over a variety of chymase concentrations, SDS-gel analyses of reaction products showing a limited number of hydrolytic products, and sequence analysis of cleavage products indicating that both inhibitors are inactivated by hydrolysis within the reactive loop, suggest that the inhibitor degradation reaction is specific and possibly linked in some fixed manner to the proteinase inactivation reaction. Reactions of serpins with proteinases that exhibit linear titrations with endpoints greater than unity, and that show the concurrent production of inactive inhibitor-proteinase complexes as well as inactivated inhibitor, have been reported for the reaction of antithrombin III with thrombin, factor Xa, and IXa (37, 38), and for the reaction of $\alpha_1$-AC with human pancreatic elastase II (38), porcine pancreatic elastase (39), cathepsin G (40), and bovine chymotrypsin (41). Except for the reaction of $\alpha_1$-AC with porcine elastase which exhibited a titration endpoint of 5.5 (39), most endpoints were at an I/E ratio less than 2. The endpoints of 4.5 and 5.0 (somewhat higher in low salt) observed for the titration of chymase with $\alpha_1$-AC and $\alpha_1$-PI, respectively, suggest that this enzyme is one of least suited proteinases for inhibition by these serpins.

Even though the serpin inhibitors $\alpha_1$-AC and $\alpha_1$-PI reversibly inactivate chymase, the slow apparent rates of chymase inactivation compared to other proteinases, and the observation that these inhibitors are better substrates for chymase than they are inhibitors strongly suggests that the regulation of chymase may not be a primary function of plasma. Tryptase, another human mast cell serine proteinase, is not inhibited by plasma (42, 43). Its activity appears to depend on a self-denaturation process which is regulated by interaction of the enzyme with heparin (44). Thus released serine proteinases from human mast cells may not be regulated in the same fashion as proteinases released from neutrophils. Possibly, these proteinases have extracellular roles that require their slow inactivation or that there are tissue inhibitors that rapidly inactivate these proteinases. Rat mast cells contain an inhibitor, termed trypstatin, which presumably regulates rat tryptase and possibly rat chymase activity (45), and bovine mast cells are the major source of bovine pancreatic trypsin inhibitor (46). Inhibitors comparable to these have not been so far identified in human mast cells.

The findings described here may not be extended to mast cell chymases of other species. Travis et al. (47) have shown that a chymotrypsin-like protease isolated from dog mastocytoma tissue was inactivated by human $\alpha_1$-AC. SDS-gel analysis of the products produced in a reaction with excess inhibitor did not contain a noticeable amount of a lower M, product indicative of degraded inhibitor. In preliminary studies, we have titrated purified dog chymase isolated from normal dog skin with human $\alpha_1$-AC (4) and have found an endpoint of 1.6, which is much lower than that obtained for human chymase. This indicates that $\alpha_1$-AC may be more effective against dog chymase than human chymase. Caution should be taken when extrapolating mechanisms of regulation to different species, however, because proteinases as well as inhibitors may have evolved different functions. An example of this has been described for a plasma inhibitor. Comparison of cDNA structure between mouse contraspisin, a trypsin inhibitor, and human $\alpha_1$-AC, a chymotrypsin inhibitor, indicate
both are evolutionary counterparts that have developed different specificities by divergence within the reactive loop region (48).

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