Inhibition of Human Chymase by Suramin

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ABSTRACT—Chymase is a chymotrypsin-like protease localized in mast cells in complexes with heparin. In the present study, we demonstrated that suramin, a hexasulfonated naphthylurea used as an anti-cancer drug, inhibits the activity of purified human chymase in vitro. The inhibition was ionic-strength-dependent. It was observed that suramin competed with heparin-Sepharose gel for binding to chymase and the inhibition of chymase activity by suramin was partially impaired by heparin. Our results show that suramin may become a prototype of a new type of chymase inhibitor because of its unique character.

Keywords: Chymase, Suramin, Heparin

Suramin, a hexasulfonated naphthylurea, was originally developed as an anti-trypanosomal agent in the 1920s (1, 2). Recently, many therapeutically significant properties of this compound have been reported, and it has proven to be effective as an anti-tumor drug primarily in patients with prostate and adrenocortical cancer (2).

Chymase [EC 3.4.21.39] is a serine protease with chymotryptic activity and is one of the most abundant proteins in mast-cell secretory granules. Because chymase is a positively-charged basic protein, it binds to heparin, which is also stored in granules (3). Although the precise pathophysiological roles of and actual substrate(s) for chymase have not as yet been determined, most recent studies have focused on the function of chymase as an angiotensin II-forming enzyme (4, 5) and it has been presumed that the inhibition of chymase activity is of therapeutic importance.

Recently, suramin was found to inhibit three human neutrophil serine proteases: neutrophil elastase, cathepsin G and proteinase 3 (6). It is worth noting that, like chymase, they all have positive net charges and heparin-binding properties. In the present in vitro experiment, we studied the inhibitory action of suramin on human chymase.

Human chymase was purified from the gastoroepiploic arteries by the procedures described previously (7). Human gastoroepiploic arteries were obtained from the surgically resected stomachs of patients who underwent total gastrectomy because of gastric cancer. Tissues were minced and homogenized in 10 vol (w/v) of 20 mM Na-phosphate buffer (pH 7.4). The homogenate was centrifuged at 20,000×g for 30 min. The supernatant was discarded, and the pellets were re-suspended and homogenized in 5 vol (w/v) of 10 mM Na-phosphate buffer (pH 7.4) containing 2.0 M KCl and 0.1% (v/v) Nonidet P-40. The homogenate was stored overnight at 4°C, and centrifuged at 20,000×g for 30 min. The supernatant was applied onto a heparin affinity column (5 ml, HiTrap Heparin; Pharmacia, Upplands, Sweden), which was pre-equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 M KCl. The column was subjected to a linear gradient (0–100%) of 20 mM Tris-HCl buffer (pH 8.0) containing 1.8 M KCl and 0.1% (v/v) Triton X-100. The active fractions from the heparin affinity column were pooled, applied onto a gel-filtration column (HiPrep 16/60 Sephacyrl S-100, Pharmacia) and then eluted by 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 M KCl and 0.1% (v/v) Triton X-100. The active fractions from the gel-filtration column gave a single band of 30 kDa on SDS-PAGE.

Chymase activity was determined using the synthetic substrate succinyl-alanyl-alanyl-prolyl-phenylalanine-4-methyl-coumaryl-7-amide (Suc-Ala-Ala-Pro-Phe-MCA; Peptide Institute, Minoh, Osaka). Unless otherwise stated, 8 ng of chymase was incubated with 0.5 mM Suc-Ala-Ala-Pro-Phe-MCA for 10 min at 37°C in 100 µl of 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 M KCl and 0.1% (v/v) Triton X-100. The reaction was terminated by the addition of 2 ml of a blocking solution consisting of 100 mM ClCH₂COONa, 30 mM CH₃COONa,
and 70 mM CH₃COOH, followed by recording the fluorescence intensity to calculate the amount of 7-amino-4-methyl-coumarin (AMC) released using excitation with emission wavelengths of 380 and 460 nm, respectively. One unit of chymase activity was defined as 1 μmol of AMC released/min. Suc-Ala-Ala-Pro-Phe-MCA was dissolved in dimethyl sulfoxide whose final concentration in the reaction medium was 5% (v/v). As a blank, Suc-Ala-Ala-Pro-Phe-MCA was incubated in the absence of chymase under the identical conditions. The results were expressed as the mean ± S.D. Suramin inhibited chymase activity in dose-dependent manner, and the IC₅₀ under this condition was calculated to be 120±11 μM (Fig. 1 (■) and Fig. 3 (■)). However, the inhibitory concentration was quite high, while much lower concentrations of suramin have been shown to inhibit a variety of enzymes, including human neutrophil elastase and cathepsin G (1, 2, 6).

Chymase is in a macromolecular complex with heparin proteoglycan or with heparan-sulfate proteoglycan in tissues, and the complex form is thought to have certain physiological significance (3). It has been well established that chymase binds to heparin by means of the electrostatic interaction between the sulfate groups of heparin and the positively charged side chains of Lys and Arg residues of the enzyme (3). Suramin is also a poly-anionic compound and thus seems to behave like heparin. Accordingly, interaction between suramin and neutrophil elastase or cathepsin G was said to be electrostatic in nature (6). The concentration of KCl in the incubation medium was varied from 0.1 to 4.0 M so that we could study the influence of ionic strength on the inhibitory activity of suramin on chymase. High concentration of KCl enhanced chymase activity, that is, activity at 0.1, 0.5, 2.0 and 4.0 M KCl were 16.12±0.98, 15.08±0.64, 29.62±1.58 and 61.52±1.92 μU, respectively. The inhibition of chymase by suramin was remarkably attenuated as the KCl concentration was increased (Fig. 1). NaCl had the same effect, but sucrose did not (data not shown). On the contrary, the inhibition of chymase by chymostatin was not attenuated by the increase in KCl concentration (data not shown). The present result suggests that electrostatic interaction may also play an indispensable role in the inhibitory mechanism of suramin on human chymase. It was thought that suramin can bind with basic amino acid residues on the surface of the chymase molecule and consequently inhibits proteolytic activity.

![Fig. 1](image1.png)

**Fig. 1.** Effect of increasing concentration of KCl on inhibition of chymase activity by suramin. Aliquots of chymase were incubated in a medium that contained 0.1 (■), 0.5 (□), 2.0 (●) and 4.0 M (○) KCl and various concentrations of suramin. The initial rate was determined using a synthetic substrate. Each point represents the mean of results from three experiments and the vertical bars represent S.D. Ordinates: ratio of initial rate in the presence (v) and in the absence of suramin (v₀).

![Fig. 2](image2.png)

**Fig. 2.** Elution of chymase from heparin-Sepharose gel by suramin. Aliquots of human chymase were first adsorbed on heparin-Sepharose gel in a buffer with low salt concentration. The gel was subjected to 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 M KCl, 0.1% (v/v) Nonidet P-40, and suramin ranging from 0–10 mM (open column). As a control, 20 mM Tris-HCl buffer (pH 8.0) containing 2.0 M KCl and 0.1% (v/v) Nonidet P-40 was added to the gel (closed column). Excess suramin in the eluent was removed by gel-filtration column chromatography, and then the chymase activity was determined. Ordinates: % of recovered total activity of chymase compared with the initial total activity.
We also examined whether suramin might interfere with the binding between heparin and chymase. Fifty microliters of heparin-Sepharose gel (Pharmacia) was suspended in 250 µl of 20 mM Tris-HCl buffer (pH 8.0) containing 80 ng of chymase. The suspension was incubated at room temperature for 10 min and centrifuged at 500 × g for 5 min. The medium was discarded and 300 µl of 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 M KCl, 0.1% (v/v) Nonidet P-40, and 0-10 mM suramin were added to the gel. As a control, an equal volume of 20 mM Tris-HCl buffer (pH 8.0) containing 2.0 M KCl and 0.1% (v/v) Nonidet P-40 was added. This mixture was incubated at room temperature for 20 min and then centrifuged at 500 × g for 5 min. Three hundred microliters of supernatant were recovered. In order to remove excess suramin, the supernatant was applied onto a PD-10 gel-filtration column (Pharmacia), which was pre-equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 2.0 M KCl and 0.1% (v/v) Nonidet P-40. The column was eluted using the identical buffer, and protein fractions were collected to determine chymase activity. The results are shown in Fig. 2. Chymase activity, which had been adsorbed on the heparin-Sepharose gel in a medium with low KCl concentration, was almost completely recovered from the gel in a buffer containing 2 M KCl, but only 2% was recovered in a buffer containing 0.5 M KCl. However, more chymase activity was recovered as the concentration of suramin was increased. More than 80% of the initial activity was recovered when the suramin concentration was increased up to 10 mM (Fig. 2). Our result suggests that suramin competes with heparin for the binding with chymase. To date, such an interesting characteristic has never been reported for chymase inhibitors known so far.

To examine whether heparin interferes with the inhibition of chymase by suramin, 0.1-100 µg of heparin (from porcine intestinal mucosa; Calbiochem, La Jolla, CA, USA) was added to the incubation medium, and the results are shown in Fig. 3. Heparin diminished the magnitude of suramin inhibition to some extent. Especially, inhibition observed at a relatively low concentration of suramin was entirely canceled. It may be that chymase activity in the presence of a higher concentration of suramin can also be completely recovered if a much higher concentration of heparin is applied, but this point remains to be elucidated. Heparin itself had no effects on the activity of human chymase (data not shown).

A chymase inhibitor with sufficient efficacy in vivo has been expected to be developed in order to demonstrate the pathophysiological relevance of chymase and to prevent progression of diseases. Although several compounds have been reported to inhibit chymase activity, none of them are in clinical use as yet. It is generally accepted that aldehyde and ketone inhibitors such as chymostatin or boronic acid inhibitors interact with the active-site serine and reversibly inhibit chymase activity. Sulfonyl fluoride inhibitors sulfonylate the active site serine and chloromethyl ketone derivatives alkylate the active site histidine; therefore, they can irreversibly inhibit chymase (8). Diphenyl phosphate derivatives that generate phosphorylated enzyme are known as another group of potent irreversible inhibitors (9). Some fatty acids inhibit chymase, and this inhibition is considered to be mediated by hydrophobic interactions (10). This is the first report, to our knowledge, that demonstrated that chymase can also be inhibited through an electrostatic interaction.

In conclusion, it became obvious that suramin itself does not have sufficient potency as a chymase inhibitor for clinical use due to its poor specificity. Furthermore, severe side-effects and its narrow therapeutic window may restrict the clinical usage of suramin (1, 2). However, it can be said that suramin may become a prototype of a new type of chymase inhibitor and provide us with meaningful information on chemical designing of unique chymase inhibitors that exhibit therapeutic efficacy.

REFERENCES
