Human Mast Cell Tryptase Activates Single-chain Urinary-type Plasminogen Activator (Pro-urokinase)*

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Human lung mast cell tryptase is a trypsin-like serine proteinase that is stored in mast cell granules and released by activated mast cells. Here we report that mast cell tryptase is a potent activator of single-chain urinary-type plasminogen activator (scu-PA, or pro-urokinase), the zymogen form of urinary-type plasminogen activator (u-PA). Activation was complete within 75 min using an enzyme:substrate molar ratio of 1:50 and was accompanied by cleavage of scu-PA at Lys158-Ile159, generating active two-chain u-PA. The reaction was dependent on enzyme concentration and obeyed Michaelis-Menten kinetics. Kinetic constants calculated for scu-PA activation by mast cell tryptase are $K_m = 34 \mu M$, $V_{max} = 3.8 \text{ pmol of u-PA/min}$, and $K_{cat} = 0.08 \text{ s}^{-1}$. These data suggest that tryptase from tumor-associated mast cells may participate in the activation of scu-PA.

Plasminogen activators (PA) catalyze the activation of the plasma zymogen plasminogen to the serine proteinase plasmin. Plasminogen mediates many physiologic and pathologic processes involving remodeling or degradation of the extracellular matrix including fibrinolysis, ovulation, wound healing, embryogenesis, neuronal migration, and tumor metastasis (for a review, see Dano et al., 1985). There are two physiologic activators of plasminogen, tissue-type PA (t-PA) and urinary-type PA (u-PA, or urokinase). Although t-PA and u-PA are distinct gene products that differ in molecular structure, both proteins are serine proteinases that catalyze cleavage of the Arg60-Val156 bond in plasminogen, resulting in the formation of active two-chain plasmin. t-PA is synthesized by vascular endothelial cells and binds with high affinity to fibrin, an association that results in marked enhancement of t-PA activity (Matsuo et al., 1981; Rijken et al., 1982). These data suggest that t-PA is primarily responsible for physiologic fibrinolysis. Alternatively, u-PA is synthesized by several cell types including kidney cells and numerous neoplastic cells (Dano et al., 1985). In contrast to t-PA, u-PA is active both in solution phase and when bound to its specific cell surface receptor, indicating that this activator is associated with processes involving cell migration, extracellular matrix degradation, and tissue invasion (Dano et al., 1985).

U-PA is secreted as a single-chain zymogen form (pro-u-PA, single-chain u-PA, or scu-PA) with a molecular mass of 55 kDa (Nolan et al., 1977; Skriver et al., 1982; Wan et al., 1982). The zymogen has low intrinsic activity against synthetic substrates and can catalyze conversion of plasminogen to plasmin at a significantly reduced rate (Gurewich et al., 1984; Lijnen et al., 1990; Ellis et al., 1989). The resulting plasmin then rapidly converts scu-PA to the fully active two chain form (tcu-PA) by cleavage at the Lys158-Ile159 bond, leading to enhanced activation of plasminogen by tcu-PA (Lijnen et al., 1987a). In addition to plasmin, activation of scu-PA by kallikrein, trypsin, T cell-associated serine proteinase, and the thioldContaining proteinase cathepsin B has been reported (Ichinose et al., 1986; Brunner et al., 1990; Kobayashi et al., 1991). In contrast, thrombin cleavage of scu-PA at an alternative site (Arg198-Phe197) generates an inactive tcu-PA derivative with greatly reduced amidolytic and plasminogen-activating activity (Ichinose et al., 1986; Gurewich and Pannell, 1987).

Tryptases are trypsin-like serine proteinases found in the cytoplasmic granules of mammalian mast cells and are stored and released as inactive enzymes (Glenner and Cohen, 1960; Alter et al., 1987). All human mast cell granules contain a tryptase, accounting for 23% of the total cellular protein (Irani et al., 1986; Schwartz et al., 1981a). Tryptases cleave peptide substrates on the carboxyl side of Lys and Arg residues, but differ from trypsin in that they have little or no activity on denatured proteins such as casein. Human tryptase activity is stabilized by heparin, an additional component of mast cell granules (Smith and Johnson, 1984; Schwartz and Bradford, 1986; Alter et al., 1987). Upon mast cell degranulation, tryptase is released along with other mediators, such as histamine, into the extracellular milieu (Schwartz et al., 1981b). There are no known inhibitors of tryptase in the human (Smith et al., 1984), and two insertion loops in tryptase, relative to trypsin, are thought to protect tryptase from blood plasma inhibitors such as α-proteinase inhibitor (Johnson and Barton, 1992). Additionally, tryptase may remain near the site of release from mast cells because it binds heparin and forms tetramers (Schwartz et al., 1981a; Smith et al., 1984).

Although the physiologic function of tryptase remains unclear, tryptase has been found to degrade fibronection, a component of the pericellular matrix that must be modified for cell migration (Lohi et al., 1992). In addition, increased levels of a tryptase-like enzyme have been found in rat mammary tumors, suggesting a role in tumor invasion (Eto and Grubbs, 1992). Mast cells have long been known to be associated with tumors (Folkmann and Shing, 1992) and mast cell-deficient mice injected with tumor cells display a significantly reduced angiogenic response (Crowle and Starkey, 1986). Together these data suggest that tryptase may participate in the proteolysis associated with tumor invasion and/or angiogenesis. As scu-PA is also found in association with numerous tumor cells, we have investigated the ability of tryptase to catalyze cleavage of scu-PA to tcu-PA. Here we report that mast cell tryptase is an
The reaction was stopped by the addition of 20 μl of Laemmli sample buffer, followed by boiling and electrophoresis on an 11% SDS-PAGE. Far right lane contains molecular weight markers: phosphorylase b, 97,600; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,400. Measurement of tcu-PA production obtained by densitometric scanning of panel A.

efficient activator of scu-PA, suggesting a mechanism by which mast cells may initiate plasmin-dependent proteolysis.

Experimental Procedures

Materials—Single-chain u-PA and two-chain uPA were purchased from American Diagnostica, Greenwich, CT. scu-PA was purchased in 100-μg aliquots and diluted to a final concentration of 1 mg/ml. Molar concentrations were determined based on a molecular weight of 55,000 (Nolan et al., 1977).

Proteins—Tryptase was purified from human lung tissue obtained at autopsy as previously described with minor modifications (Smith et al., 1984). Changes included the use of cetlyl pyridinium chloride to reduce the viscosity of the extract and Toyopearl Butyl 650M hydrophobic chromatography media was substituted for octyl-Sepharose. Tryptase used in these studies corresponded to the major isoform present in preparations from lung (Little, 1993). Tryptase concentration was determined based on the 280-nm extinction coefficient of 28.1 for a 1% solution as reported by Smith et al. (1984). Plasminogen was purified from pooled human plasma by affinity chromatography as previously described (Deutsch and Mertz, 1970; Gonzalez-Gronow and Robbins, 1984).

Activation of scu-PA by Tryptase—Because tryptase efficiently cleaves all synthetic peptide substrates normally used to monitor activation of scu-PA (data not shown), conversion of scu-PA to tcu-PA was analyzed by electrophoresis followed by densitometric scanning to quantitate reaction products. Tryptase was incubated with scu-PA at 37°C in 100 mM Hepes, 0.05% Brij 35, pH 7.5, and the reaction was stopped by the addition of Laemmli sample dilution buffer and boiling (Laemmli, 1970). Samples were electrophoresed on 11% SDS-PAGE, gels were stained with Coomassie Blue, and the bands of interest were excised, placed on Porton sample support disks, and sequenced (Matsudaira, 1987). The net yields of amino acids were calculated by subtraction of the amount of phenylthiohydantoin derivatives in each cycle from the background in the previous cycle.

Results

Incubation of scu-PA with catalytic amounts of tryptase resulted in time-dependent cleavage of the proenzyme (Fig. 1A), with 50% conversion occurring within approximately 30–40 min (Fig. 1B). The molecular weights of the reaction products were consistent with the generation of characteristic A (Mₐ = 22,000) and B (Mₐ = 33,000) chains of the active form of u-PA (Skriver et al., 1982). Amino-terminal sequence analysis of the B chain indicated a sequence of Ile-Ile-Gly-Glu-Glu-Phe-Thr-Ile-Glu-Asn-Gln, corresponding to amino acids 159–170 of u-PA (Holmes et al., 1985). The resulting active tcu-PA catalyzed the conversion of plasminogen to two-chain plasmin (data not shown). Under identical conditions, no direct activation of plasminogen by tryptase was observed (data not shown). At low concentrations of tryptase (greater than 25-fold molar excess of the substrate scu-PA), scu-PA activation was a linear function of tryptase concentration (Fig. 2, A and B). The kinetics of scu-PA activation were determined by incubating increasing concentrations of scu-PA (2.5–17.5 μM) with tryptase (0.04 μM) (Fig. 3A). The Michaelis-Menten plot shows that the activation...
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**Fig. 3. Kinetics of scu-PA activation by tryptase.** A, increasing concentrations of scu-PA were incubated with tryptase (3.7 x 10^{-5} M) for 2 h at 37 °C in a total volume of 20 μl. Reactions were stopped by the addition of 20 μl of Laemmli sample buffer and boiling, followed by electrophoresis on 11% SDS-PAGE. Molecular weight markers are as in Fig. 1. B, Michaelis-Menten plot of data from SDS-PAGE obtained by densitometric scanning of panel A. C, Lineweaver-Burk plot of scu-PA activation by tryptase. Data were obtained by densitometric scanning of panel A.

reaction is concentration dependent and saturable (Fig. 3B). Analysis of the data using a double reciprocal plot to determine kinetic constants (Fig. 3C) gave a \( k_{\text{m}} \) of 34 μM and a \( V_{\text{max}} \) of 3.6 pmol of tcu-PA generated/min. The catalytic rate constant for the reaction (\( k_{\text{cat}} \)) was 0.08 s^{-1}.

**DISCUSSION**

Previous studies have shown that cleavage of scu-PA at the Lys^{158}-Ile^{159} bond by serine proteinases including plasmin and kallikrein generates the proteolytically active two-chain form of the molecule, which is a potent plasminogen activator (Lijnen et al., 1987b; Ichinose et al., 1986). The identical activation cleavage is also catalyzed by the thiol proteinase cathepsin B (Kobayashi et al., 1991). Alternatively, cleavage at Arg^{156}_Hyp^{157} by thrombin generates a catalytically inactive tcu-PA derivative (Ichinose et al., 1986; Gurewisch and Pannell, 1987). The present study demonstrates that activation of scu-PA can also be initiated by human mast cell tryptase. Comparison of the kinetic constants for scu-PA activation by tryptase and plasmin indicates a decreased catalytic efficiency for the tryptase-catalyzed reaction, resulting from both an increase in \( K_{\text{m}} \) and a decrease in \( k_{\text{cat}} \) (tryptase \( K_{\text{m}} = 34 \mu M, k_{\text{cat}} = 0.08 s^{-1}; \) plasmin \( K_{\text{m}} = 7.1 \mu M, k_{\text{cat}} = 1.3 s^{-1} \)) (Lijnen et al., 1987a). Although no kinetic data are available for the kallikrein-catalyzed reaction, the activation follows a similar time course as observed for tryptase, with activation complete within 2 h (using an enzyme: substrate ratio of 1:30 (w/w)) (Ichinose et al., 1986).

Many studies have demonstrated an increase in mast cells, both intact and degranulated, in the vicinity of tumors (Crowle and Starkey, 1989). While mast cell degranulation resulting from antigen binding by cell surface-IgE is well recognized, other factors such as hypoxia, neurotransmitters, opiates, factors derived from other cells, and even physical stimuli can serve as mast cell secretagogues (Friedman and Kaliner, 1987; Wasserman, 1990). Thus, it is likely that tumor-associated mast cells release tryptase in response to various stimuli. Plasminogen activators, particularly u-PA, have also been found in association with numerous invasive and metastatic cells and in a variety of tumor tissues (Dano et al., 1985). In addition, recent data indicate that scu-PA is the predominant molecular form in cultures of adherent tumor cells (TapiоЬareta et al., 1993). The present data demonstrate that human mast cell tryptase is a potent activator of scu-PA. In contrast to other activators of scu-PA such as plasmin, kallikrein, and cathepsin B, which are rapidly inactivated by endogenous proteinase inhibitors, tryptase is remarkably resistant to inhibition by plasma and other proteinase inhibitors (Smith et al., 1984). Although the significance of our findings relative to other activation pathways will require additional study, the possibility that tryptase retains activity in the extracellular milieu, coupled with its ability to activate scu-PA, suggests a previously undescribed role for tryptase in the initiation and/or amplification of tumor-associated proteolytic activity.

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