Cellular Internalization and Degradation of Antithrombin III-Thrombin, Heparin Cofactor II-Thrombin, and α1-Antitrypsin-Trypsin Complexes Is Mediated by the Low Density Lipoprotein Receptor-related Protein*

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The inhibition of proteinase activity by members of the serine protease inhibitor (serpin) family is a critical regulatory mechanism for a variety of biological processes. Once formed, the serpin enzyme complexes (SECs) are removed from the circulation by a hepatic receptor. The present study suggests that this receptor is very likely the low density lipoprotein receptor LRP (a prominent liver receptor). 

In vitro binding studies revealed that antithrombin III (ATIII)-thrombin, heparin cofactor II (HCII)-thrombin, and α1-antitrypsin (α1AT)-trypsin bound to purified LRP, and their binding was inhibited by the 39-kDa receptor-associated protein (RAP), an antagonist of LRP-ligand binding activity. In contrast, native or modified forms of the inhibitors were unable to bind to LRP. Mouse embryonic fibroblasts, which express LRP, mediate the cellular internalization leading to degradation of these SECs, while mouse fibroblasts genetically deficient in LRP showed no capacity to internalize and degrade these complexes. SECs were also degraded by HepG2 cells, and this process was inhibited by LRP antibodies, RAP, and chloroquine. The cellular-mediated uptake and degradation was specific for SECs; native or modified forms of the inhibitors were not internalized and degraded. Finally, in vivo clearance studies in rats demonstrated that RAP inhibited the clearance of ATIII-thrombin complexes from the circulation.

Together, these results indicate that LRP functions as a liver receptor responsible for the plasma clearance of SECs.

Serine proteinase inhibitors (serpins) are a supergene family comprised of more than 40 members (Carrell and Travis, 1985). Most serpins function to inhibit serine proteinases through the formation of a stable serpin-proteinase complex (SEC).

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The abbreviations used are: SEC, serpin-enzyme complex; LDLR, low density lipoprotein receptor; LRP, LDLR-related protein; gp330, glycoprotein 330; RAP, receptor-associated protein; ATIII, antithrombin III; HCII, heparin cofactor II; α1AT, α1-antitrypsin; PAI-1, plasminogen activator inhibitor type-1; uPA, urokinase-type plasminogen activator; α2M, α2-macroglobulin; αM*, methylamine-activated αM; PR3, proteinase 3; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; TBS, Tris-buffered saline; MEF, mouse embryonal fibroblast.
Suggesting that HCII, like ATIII, is an important inhibitor of thrombin in vivo.

An important feature of serpin metabolism was originally identified when Lollar et al. (1980) showed that 125I-thrombin in complex with ATIII was rapidly cleared from the circulation of rabbits by the liver. Additional studies by several groups (Bauer et al., 1982; Fuchs et al., 1982; Pratt et al., 1988) have demonstrated that the liver mediates rapid clearance of complexes of ATIII-thrombin, HCII-thrombin, AT-thrypsin, and AT-elastase from the circulation. Further, these complexes could compete for one another’s clearance, indicating that the plasma elimination of these SECs was occurring via a common receptor pathway. Mast et al. (1991) showed that the kinetics of plasma elimination of the native and cleaved inhibitors were 10-50 times slower than that of the complexed inhibitors, suggesting that the liver receptor might preferentially clear the SEC as opposed to the free serpin.

The low density lipoprotein receptor (LDLR) and low density lipoprotein-related protein (LRP) are two prominent hepatic receptors that are members of the LDLR superfamily. This receptor family also includes the very low density lipoprotein receptor and glycoprotein 330 (gp330). LRP is expressed in many tissues and cell types (Moestrup et al., 1992) and is present in high abundance in the liver. The requirement for LRP expression during development was demonstrated when targeted disruption of the LRP gene resulted in termination of the null embryos at day 13.5 during development (Herz et al., 1992). LRP interacts with numerous ligands and is thought to play key roles in both lipoprotein metabolism and proteinase regulation (for reviews, see Krieger and Herz (1994), Strickland et al. (1994, 1995), and Moestrup (1994)).

In addition to mediating the catabolism of α2-macroglobulin (α2M)-proteinase complexes (Ashcom et al., 1990), LRP also binds to several SECs, including elastase-AT (Poller et al., 1995), urinary-type plasminogen activator (uPA)-PAI-1 (Herz et al., 1992; Nykjaer et al., 1992), and tissue plasminogen activator-PAI-1 (Orth et al., 1992). However, the receptor responsible for the hepatic catabolism of a number of other predominant SECs remained obscure. Studies were therefore initiated to determine if LRP could interact with those SECs. In this report we demonstrate that LRP binds directly to complexes of ATIII-thrombin, HCII-thrombin, and AT-thrypsin, can mediate their cellular uptake and degradation, and appears responsible for their in vivo clearance from the circulation.

**EXPERIMENTAL PROCEDURES**

**Proteins—**LRP was isolated from human placenta as described by Ashcom et al. (1990). Human RAP, expressed in bacteria as a fusion protein with glutathione S-transferase, was prepared and purified as described previously (Williams et al., 1992). α2M was purified from human plasma by zinc-chelate chromatography as described previously (Ashcom et al., 1990). α2M-methylamine, designated (α2M-Ma), was prepared by incubation of native α2M with 200 mM methylamine (Sigma) for 30 min at 25°C followed by dialysis against Tris-buffered saline (TBS). HCII was purified from human plasma as described previously (Griffith et al., 1985). Antithrombin III was isolated from human plasma as described (Mille-Andersson et al., 1974). AT was purchased from Athens Research and Technology (Athens, GA). Inhibitors inactivated by cleavage of the reactive center loop, without complex formation, were added to as modified as possible inhibitor preparations. Inhibitors were prepared by incubation of inhibitor in TBS (1 mg/ml) with 100 μl of activated immobilized papain-Sepharose (Pharoc) for 30 min at 25°C. To monitor the efficiency of papain to cleave inhibitors, cleaved preparations were tested for their ability to inhibit target proteases. In addition, SDS-polyacrylamide gel electrophoresis analysis of the inhibitors revealed a shift in mobility on 8-16% polyacrylamide gradient gels (Novex, San Diego, CA) as compared with native inhibitors and also showed that papain treatment did not result in excess proteolysis of the cleaved inhibitors. Thrombin (α-form) was purchased from Enzyme Research Laboratories (South Bend, IN). Trypsin was purchased from Sigma. To prepare complexes of ATIII-thrombin and HCII-thrombin, the enzyme and inhibitor were combined in a 1:2 molar ratio and were incubated for 30 min at 25°C. Complexes of α2AT-thrypsin were prepared by incubation of trypsin and α2AT at a 1:2 molar ratio for 5 min at 25°C, followed by addition of soybean trypsin inhibitor (Saxima) (in a molar amount equal to that of trypsin added) to inhibit any residual trypsin activity. uPA was provided by Jack Henkin (Abbott Park, IL). Active PAI-1 was purchased from Molecular Innovations (Royal Oak, MI). Complexes of uPA-PAI-1 were prepared by incubation of a 1:1 molar ratio of active PAI-1 with uPA for 30 min at 25°C. Proteins were labeled with [125I]iodine to a specific activity ranging from 10 to 100 μCi/μg of protein (in a molar amount equal to that of IODIGEN (Pierce) added). Bovine serum albumin, fraction V (BSA) was purchased from Sigma.

**Antibodies—**A rabbit polyclonal antibody to the 515-kDa heavy chain of LRP (R777) and another one to a synthetic peptide from the cytoplasmic domain of the 85-kDa light chain of LRP (R704) have been described elsewhere (Kounnas et al., 1992). R777 antibodies were affinity-purified on a column of LRP-Sepharose. R704 antibodies were purified on protein G-Sepharose (Pharmacia Biotech Inc.). Both of the rabbit antibody preparations were dialyzed against isotonic PBS, heat-inactivated for 30 min at 56°C, and filtered with a 0.45 μm Acrodisc (Corning, Corning, NY) prior to use in cell assays. The mouse monoclonal antibody to LRP, designated BGl, has been described (Strickland et al., 1990).

**Solid-Phase Binding Assays—**Enzyme-linked immunosorbent assays (ELISA) were performed as detailed elsewhere (Kounnas et al., 1993). Briefly, microtiter wells were coated with the native, cleaved, or complexed forms of ATIII, HCII, or α2AT (prepared as described under “Proteins”) at 10 μg/ml in TBS, pH 8.0, for 4 h at 37°C, blocked with 3% BSA, TBS, pH 8.0, then incubated with various concentrations of LRP (0.6-150 nM) in 3% BSA, TBS, 5 mM CaCl2, 0.05% Tween-20 for 18 h at 4°C. For ELISAs measuring the effects of RAP, coated wells were incubated with 200 nM LRP in the presence of increasing concentrations of RAP (0.2-450 nM). LRP binding was detected with the mouse monoclonal antibody BGl.

**Cdl—**Human hepatocellular carcinoma (HePG2, ATCC HB 8065) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were grown in Eagle's minimal essential medium containing 10% bovine calf serum (HyClone Laboratories, Logan, UT), 100 units/ml penicillin (Life Technologies, Inc.), 100 μg/ml streptomycin (Life Technologies, Inc.), and 1% l-glutamine (Life Technologies, Inc.). A normal mouse embryonal fibroblast line (MEF) and a mouse embryonal fibroblast cell line that is deficient in LR biosynthesis (PEA13) were obtained from Dr. Joanne Jeruss (University of Texas Southwestern Medical Center, Dallas, TX) and maintained as described by Willnow and Herz (1994).

**Cdl-mediated Ligand Internalization and Degradation Assays—**Cells were seeded into 12-well dishes (Corning) at 2-3 x 104 cells/well and allowed to grow for 24 h at 37°C, 5% CO2. Cellular internalization and degradation assays were conducted according to Kounnas et al. (1995a). Prior to performing the assays, cultured cells were washed with medium and incubated in medium containing 1% Nutridoma (Boehringer Mannheim), 20 mM Hepes, penicillin/streptomycin, and 1.5% BSA (assay medium). For assays with HePG2 cells, radiolabeled serpin complexes (10 nM) in assay medium were incubated with cells for 18 h at 37°C in the presence of RAP (1 μM), LRP antibodies (100 μg/ml), or chloroquine (0.1 mM, Sigma). For assays using antibodies, cultured cells were preincubated for 1 h at 37°C with assay medium containing antibodies. Antibodies (100 μg/ml) were also included during the cellular assays. For assays utilizing cultured fibroblasts, 125I-proteins (5-10 nM) were added in assay medium either alone or in the presence of RAP (1 μM) for 1 hour (indicated time). The radioactive activity secreted in the cell culture medium that was soluble in 10% trichloroacetic acid was corrected for non-cellular mediated degradation by subtracting the amount of degradation that occurred in parallel wells lacking cells. The amount of radiolabeled ligand that was internalized by cells was defined as the amount of radioactivity that remained associated with the cell pellet following tryptic-EDTA, proteinase K (Sigma) treatment (Chappell et al., 1992).

**Clearance of ATIII—**Clearance of ATIII-Thrombin from the Plasma of Rats—Sprague-Dawley rats (200 g) were anesthetized with ketamine (100 mg/ml)/xylazine (20 mg/ml) at a dose of 90 mg/kg ketamine, 8 mg/kg xylazine. A bolus of 500 μl of ATIII-Thrombin (100 nM) in the presence or absence of RAP (1 μM) was injected into the tail vein over a period of approximately 15 s. At selected time intervals following injection (1, 5, 10, and 20 min), blood (200 μl) was collected from the vena cava into 10 μl of 0.5 M EDTA, and an aliquot (50 μl) was counted for its 125I content.
Fig. 1. Binding of LRP to ATIII-thrombin, HCII-thrombin, and αAT-trypsin. In panel A, increasing concentrations of LRP (0.6–150 nM) were incubated for 18 h at 4 °C with wells coated with native ATIII (○), cleaved ATIII (●), ATIII-thrombin (■), or BSA (□). Panel B, native HCII (○), cleaved HCII (●), HCII-thrombin (■), and BSA (□). Panel C, native αAT (○), cleaved αAT (●), αAT-trypsin (■), and BSA (□). Following incubation, wells were washed and bound LRP detected with the LRP monoclonal antibody 8G1.

Fig. 2. RAP inhibits the binding of LRP to complexes of ATIII-thrombin, HCII-thrombin, and αAT-trypsin. Wells coated with ATIII-thrombin (△), HCII-thrombin (■), αAT-trypsin (●), or BSA (□) were incubated with 20 nM LRP in the presence of increasing concentrations of RAP (0.2–450 nM) for 18 h at 4 °C. Following incubation, wells were washed and bound LRP detected with the LRP monoclonal antibody 8G1.

In those experiments examining the clearance of 125I-125I-thrombin, 125I-trypsin, and 125I-BSA (see Fig. 2). The ability of RAP to block the binding of LRP—The ability of LRP to bind to native, cleaved, or complexed forms of ATIII, HCII, and αAT was measured using an ELISA. As shown in Fig. 1, LRP binds to ATIII-thrombin (panel A), HCII-thrombin (panel B), and αAT-trypsin (panel C), while no detectable binding to the native or cleaved forms of ATIII (panel A), HCII (panel B), and αAT (panel C) was observed. The lack of saturation of these assays indicates that the affinity of LRP for these SECs is weaker than that measured for other LRP ligands. Apparent half-saturation values measured for the binding interaction of these complexes with LRP are estimated to range from 80 to 120 nM.

Since RAP is known to inhibit the binding of ligands to LRP (Herz et al., 1991), binding assays were performed to test the effect of RAP on the binding of LRP to ATIII-thrombin, HCII-thrombin, and αAT-trypsin. Fig. 2 depicts an ELISA in which LRP was incubated with serpin-proteinase-coated wells in the presence of increasing concentrations of RAP. These data demonstrate that RAP is capable of competing for the binding of these SECs to LRP.

Efficient Uptake and Degradation of Complexes of 125I-Thrombin and 125I-Trypsin in HepG2 Cells—To further implicate LRP in mediating the uptake and degradation of ATIII-125I-thrombin, HCII-125I-thrombin, and αAT-125I-trypsin in HepG2 cells, the uptake and degradation of labeled complexes of 125I-thrombin, 125I-trypsin, and 125I-BSA (see Fig. 2). The ability of RAP to block uptake and degradation of 125I-SECs in LRP-expressing cells and the inability of LRP-deficient cells to mediate SEC uptake and degradation point to LRP as a mediator of the endocytosis of SECs.

LRP Antibodies Inhibit the Degradation of ATIII-125I-Thrombin, HCII-125I-Thrombin, and αAT-125I-Trypsin in MEF Cells. Since RAP is known to inhibit the binding of ligands to LRP (Herz et al., 1991), binding assays were performed to test the effect of RAP on the binding of LRP to ATIII-thrombin, HCII-thrombin, and αAT-trypsin. Fig. 2 depicts an ELISA in which LRP was incubated with serpin-proteinase-coated wells in the presence of increasing concentrations of RAP. These data demonstrate that RAP is capable of competing for the binding of these SECs to LRP.

RESULTS

ATIII-Thrombin, HCII-Thrombin, and αAT-Trypsin Bind to LRP—The ability of LRP to bind to native, cleaved, or complexed forms of ATIII, HCII, and αAT was measured using an ELISA. As shown in Fig. 1, LRP binds to ATIII-thrombin (panel A), HCII-thrombin (panel B), and αAT-trypsin (panel C), while no detectable binding to the native or cleaved forms of ATIII (panel A), HCII (panel B), and αAT (panel C) was observed. The lack of saturation of these assays indicates that the affinity of LRP for these SECs is weaker than that measured for other LRP ligands. Apparent half-saturation values measured for the binding interaction of these complexes with LRP are estimated to range from 80 to 120 nM.

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αAT-125I-trypsin complexes, the effect of LRP antibodies on the degradation of 125I-labeled SECs by HepG2 cells was determined. Fig. 5 shows that RAP, LRP antibodies, and chloroquine (a drug that blocks lysosomal degradation) inhibit the degradation of ATIII-125I-thrombin (panel A), HCII-125I-thrombin (panel B), and αAT-125I-trypsin (panel C). In each case, control antibodies had little effect. These data demonstrate that LRP is capable of mediating the endocytosis leading to lysosomal degradation of labeled ATIII-thrombin, HCII-thrombin, and αAT-trypsin in HepG2 cells, raising the possibility that LRP can function in vivo to mediate the liver clearance of these complexes.

The Uptake and Degradation of 125I-ATIII, 125I-HCII, and 125I-αAT Requires Complex Formation with an Enzyme—Previous studies examining the clearance of 125I-labeled SECs from plasma in a mouse model have shown that while the complexed form of ATIII, HCII, and αAT are cleared rapidly by hepatocytes, the clearance of the native and cleaved inhibitors is slow (Mast et al., 1991). We, therefore, performed assays to investigate the LRP-mediated cellular endocytosis of 125I-labeled inhibitors in the native, cleaved, or complexed forms. Fig. 6 shows the uptake of 125I-ATIII (native, cleaved, and thrombin complexed) in MEF (LRP-expressing cells) (panel A) and PEA13 (LRP-deficient cells) (panel B), 125I-HCII (native, cleaved, and thrombin complexed) in MEF and PEA13 cells (panels C and D, respectively), and 125I-αAT (native, cleaved, and complexed to trypsin) in MEF and PEA13 cells (panels E and F, respectively). These data demonstrate that the labeled inhibitors alone (native or cleaved) are not endocytosed by mouse fibroblasts. However, complexes of 125I-ATIII-thrombin, 125I-HCII-thrombin, and 125I-αAT-trypsin are internalized efficiently by LRP-expressing MEF cells but not the mutant LRP-deficient PEA13 cells and this process is inhibited by RAP. Further, these complexes are efficiently degraded in LRP-expressing cells (but not LRP-deficient cells) in a RAP-dependent manner with similar kinetics as shown in Fig. 4 (data not shown). These data reveal that protease complex formation of ATIII, HCII, and αAT is required for LRP-mediated endocytosis.

Comparative Degradation of Different LRP Ligands by Mouse Fibroblasts—Complexes of uPA-PAI-1 bind to LRP with an affinity constant of 1 nM (Nykaer et al., 1994), while complexes of ATIII-thrombin, HCII-thrombin, and αAT-trypsin bind to LRP with estimated affinities of 80–120 nM. This observation prompted us to compare the amounts of 125I-labeled SECs that were degraded by cells in an LRP-dependent manner. The first experiments were designed to determine the relative levels of 125I-labeled SEC degraded by mouse fibro-

![Fig. 4. LRP-deficient fibroblasts do not internalize and degrade complexes of 125I-ATIII, 125I-HCII, and 125I-trypsin-αAT. Wells containing 2 × 10⁵ MEF (LRP-expressing) or PEA13 (LRP-deficient) cells were incubated with 125I-enzyme-serpin complexes (50 nm) for selected time intervals at 37 °C in the presence and absence of RAP (1 μM). At the indicated times, the amount of radioligand internalized by the cell and the levels of degraded radioligand secreted into the cell medium were determined as described under “Experimental Procedures.” Panels show internalization and degradation of ATIII-125I-thrombin (A and B), HCII-125I-thrombin (C and D), and αAT-125I-trypsin (E and F), respectively. The data shown are representative of three experiments, each performed in duplicate.

![Fig. 5. LRP antibodies inhibit 125I-thrombin-ATIII, 125I-thrombin-HCII, and 125I-trypsin-αAT degradation by HepG2 cells. Wells containing 2 × 10⁵ HepG2 cells were incubated for 18 h at 37 °C with 125I-labeled complexes (30 nm) in the presence of affinity-purified anti-LRP IgG (100 μg/ml), control IgG against the cytoplasmic domain of LRP (100 μg/ml), chloroquine (0.1 mM), or RAP (1 μM). Panels show degradation data of 125I-ATIII-thrombin (A), 125I-HCII-thrombin (B), and 125I-αAT-trypsin (C). Plotted values represent means of duplicate values.
Catabolism of SECs by LRP

Effect of RAP on the Plasma Clearance of $^{125}$I-$\omega_2$M* and ATIII-$^{125}$I-Thrombin Complexes—To evaluate the role of LRP in mediating the plasma clearance of SECs, the effect of RAP on the clearance rate of a representative SEC, the ATIII-thrombin complex, was measured. RAP is known to have a pronounced effect on the removal of $^{125}$I-$\omega_2$M* from the mouse circulation (Willnow et al., 1994), and thus $^{125}$I-$\omega_2$M* was used as a control for these experiments. The results, shown in Fig. 8, demonstrate that when co-injected with the ligand, RAP significantly delays the clearance of $^{125}$I-$\omega_2$M* (Fig. 8A) and ATIII-$^{125}$I-thrombin complexes (Fig. 8B) from the rat circulation. These studies indicate that a RAP-sensitive hepatic receptor, most likely LRP, plays a major role in the removal of ATIII-$^{125}$I-thrombin complexes from the circulation.

DISCUSSION

The existence of a hepatic receptor that is responsible for binding and removing SECs from the plasma was demonstrated in early studies (Pizzo and Gonias, 1984; Shifman and Pizzo, 1982; Imber and Pizzo, 1981; Fuchs et al., 1982; Pratt et al., 1988) in which labeled SECs (ATIII-thrombin, HCII-thrombin, and AT-tryptasin, and AT-elastase) were shown to be rapidly cleared by the liver (half-lives = 3, 10, 15, and 20 min, respectively). These complexes apparently all bound to the same receptor, since they were able to cross-compete with one another (Pratt et al., 1988). However, clearance of these molecules was not competed with high concentrations of $\alpha_2$M-protease complexes (Fuchs et al., 1982). The clearance mechanism appears specific for the enzyme-complexed form of these serpins, since studies by Mast et al. (1991) demonstrated that the clearance of native and cleaved forms of ATIII, HCII, and AT occurred at a considerably slower rate (half-lives > 1 h) than the proteinase-complexed serpins.

The data presented in the current study suggest that LRP is the hepatic receptor responsible for the clearance of these complexes. This conclusion is supported by several independent lines of evidence. First of all, we demonstrated that purified LRP can directly bind to several SECs (ATIII-thrombin, HCII-thrombin, and AT-tryptasin) coated on microtiter wells, and that the binding is competed by RAP. Second, SECs are internalized and degraded in mouse fibroblasts that express LRP, but not in mouse fibroblasts genetically deficient in LRP. Third, we demonstrated that LRP antagonists, RAP and LRP antibodies, blocked the cellular uptake and degradation of $^{125}$I-labeled ATIII-thrombin, HCII-thrombin, and $\alpha_2$AT-tryptasin.

Fourth, our studies have shown that the interaction of SECs with LRP has properties expected of the hepatic clearance mechanism; LRP does not recognize the native and cleaved forms of the inhibitors but readily internalizes the proteinase-complexed form of the inhibitors. Taken together, these data suggest that the liver receptor responsible for the plasma clearance of ATIII-thrombin, HCII-thrombin, and $\alpha_2$AT-tryptasin is LRP.

To demonstrate this, in vivo clearance studies were performed in rats. The ability of RAP to inhibit hepatic clearance of ATIII-$^{125}$I-thrombin indicates that a RAP-sensitive hepatic receptor is responsible for this process. While RAP is known to bind to all members of the LDLR superfamily, only two of these receptors, LDLR and LRP, are known to be expressed in high levels in the liver. Our studies indicate that the LDLR does not play a significant role in the catabolism of SECs, since PEA13 fibroblasts, which express LDLR and are normal in their ability to catabolize LDL (Willnow and Herzer, 1994; Kounnas et al., 1995a), are unable to mediate the cellular catabolism of SECs. Thus, the in vivo clearance studies suggest that LRP is playing...
that binding of elastase to the cell surface may result in signal transduction events. Perlmutter et al. (1990a, 1990b) described a SEC receptor on monocytes and HepG2 cells which internalized complexes of α1-AT-trypsin and α1-AT-elastase, and it is highly likely that this receptor is LRP. In the present investigation, we found that the cellular uptake and degradation of ATIII-thrombin, HCII-thrombin, and α1-AT-trypsin by Hep G2 cells is inhibited by anti-LRP IgG. In addition to a receptor that mediates the endocytosis of SECs to the cell surface, signal transduction events may also be involved in the degradation of SECs. Studies by several investigators suggest that binding of SECs to the cell surface may result in signal transduction events. Perlmutter et al. (1988) demonstrated that binding of elastase-α1-AT complexes to monocytes or macrophages resulted in increased gene expression of α1-AT, while Banda et al. (1988) showed that cleaved α1-AT stimulated neutrophil chemotaxis. Hoffman et al. (1989) reported that HCII could be cleaved by proteinase 3 to generate products that stimulated neutrophil chemotaxis, suggesting that cleaved serpins release biologically active molecules. While the mechanism of signal transduction by these complexes is unclear, it seems likely that a distinct signaling receptor, different from LRP, may exist and would account for these effects.

The existence of a clearance mechanism for SECs is likely important since such complexes are known to dissociate with time, releasing active enzyme along with the cleaved inhibitor (Travis and Salvesen, 1983; Carrell and Boswell, 1986). ATIII is a primary inhibitor of thrombin and Fx, and plays a major role in maintaining normal hemostasis (Bjork et al., 1989; Olsen and Bjork, 1992). Disruption of the normal balance of thrombin and inhibitor activity results in either thrombosis (Egeberg, 1965; Lane et al., 1991) or bleeding disorders (Owen et al., 1983); therefore, it is clear that maintaining an appropriate amount of thrombin activity is critical for normal hemostasis. The principle action of ATIII may be to localize clotting factors, and help maintain normal hemostasis. The imbalance of α1-AT/proteinase activity has been associated with various pathological conditions such as pulmonary emphysema (Laurell and Eriksson, 1963; Travis and Salvesen, 1983; Carrell, 1986). α1-AT has been implicated as a primary modulator of elastase and proteinase 3 activity during inflammatory processes. In acute inflammation, infiltrating neutrophils and macrophages, as well as damaged cells, release excessive amounts of proteinases resulting in tissue damage. This damage can be minimized by rapid inhibition of proteinase activity by inhibitors present in body fluids. Elastase and PR3 are released by neutrophils during inflammation and participate in the degradation of extracellular matrix components.
Once these enzymes are inhibited by α1-AT, the complexes formed are known to be cleared by macrophages and fibroblasts in a receptor-mediated process. LRP is found in high concentration on fibroblasts and macrophages (Moestrup et al., 1992) and has been shown to bind and mediate the uptake of at least two α1-AT-proteinase complexes (Poller et al., 1995; this study) and therefore is very likely to represent the receptor responsible for the endocytosis of α1-AT-proteinase complexes formed during the inflammatory response.

Comparative experiments demonstrated that several higher affinity LRP ligands such as uPA-PAI-1 and α2M were internalized and subsequently degraded to a greater extent than complexes of ATIII-thrombin, HCII-thrombin, and α1-AT-trypsin. This is likely related to the weaker affinity of these latter ligands for LRP. Interestingly, the affinity for the interaction of ligands with LRP varies considerably: uPA-PAI-1 (<1 nm; Nykaer et al., 1994), α2M-methylamine (10 nm; Ashcom et al., 1990), LpL (18 nm; Chappell et al., 1992), thrombospondin (3–20 nm; Mikhailenko et al., 1995), pro-uPA (45 nm; Kounnas et al., 1993), PAI-1 (55 nm; Nykaer et al., 1994), hepatic lipase (52 nm; Kounnas et al., 1995a), apoS (54 nm; Kounnas et al., 1995b), amyloid precursor protein (80 nm; Kounnas et al., 1995c), and approximately 80–120 nm for the complexes of ATIII-thrombin, HCII-thrombin, and α1-AT-trypsin. The relatively lower affinity of LRP for the SECs (ATIII-thrombin, HCII-thrombin, and α1-AT-trypsin) as compared to other ligands may be compensated for during conditions of thrombosis or inflammation where the plasma or interstitial fluid levels of complexes are greatly elevated. Other factors may also augment LRP-mediated uptake of the SECs. For example cell-surface proteoglycans, which have been shown to facilitate the uptake of a number of LRP ligands including lipoprotein lipase (Chappell et al., 1993), hepatic lipase (Kounnas et al., 1995), and thrombospondin (Mikhailenko et al., 1995), may act to concentrate SECs on the surface of LRP-expressing cells and thereby increase their local concentration.

In summary, the current investigation demonstrates that LRP can bind and endocytose complexes of ATIII-thrombin, HCII-thrombin, and α1-AT-trypsin, but not the native or the degraded enzymes in vivo. Close studies confirm that LRP functions to remove SECs from the plasma. These findings expand the role of LRP as a SEC receptor.

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