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Characterization of α_2-Macroglobulin–Plasmin Complexes: Complete Subunit Cleavage Alters Receptor Recognition in Vivo and in Vitro†

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ABSTRACT: When human α_2-macroglobulin (α_2M) binds proteinases, it undergoes subunit cleavage. Binding of small proteinases such as trypsin results in proteolysis of each of the four subunits of the inhibitor. By contrast, previous studies suggest that reaction of plasmin with α_2M results in cleavage of only two or three of the inhibitor subunits. In this paper, we demonstrate that the extent of subunit cleavage of α_2M is a function of plasmin concentration. When α_2M was incubated with a 2.5-fold excess of plasmin, half of the subunits were cleaved; however, at a 20-fold enzyme to inhibitor ratio, greater than 90% of the subunits were cleaved with no additional plasmin binding. This increased cleavage was catalyzed by free rather than bound plasmin. It is concluded that this "nonproductive" subunit cleavage is dependent upon the molar ratio of proteinase to inhibitor. The consequence of complete subunit cleavage on receptor recognition of α_2M–plasmin (α_2M–Pm) complexes was studied. Preparations of α_2M–Pm with only two cleaved subunits bound to the murine macrophage receptor with a K_d of 0.4 nM and 60 fmol of bound complex/mg of cell protein. When preparations of α_2M-Pm with four cleaved subunits were studied, the K_d was unaltered but ligand binding increased to 140 fmol/mg of cell protein. The receptor binding behavior of the latter preparation is equivalent to that observed when α_2M is treated with small proteinases such as trypsin. This study suggests that receptor recognition site exposure is not complete in the α_2M–Pm complex with half of the subunits cleaved. Proteolytic cleavage of the remaining subunits of the inhibitor results in a further conformational change exposing the remaining receptor recognition sites.

Human α_2-macroglobulin (α_2M) is a glycoprotein composed of four identical ($M_r \sim 180\,000$) subunits that inhibits endopeptidases of all four major classes (Barrett & Starkey, 1973; Hall & Roberts, 1978; Swenson & Howard, 1979; Sottrup-Jensen et al., 1983). The mechanism of inhibition is unique in that specific, limited proteolysis of α_2M at a "bait region" located near the middle of each subunit (Barrett & Starkey, 1973; Harpel, 1973; Mortensen et al., 1981) results in a conformational change in the inhibitor that physically entraps the proteinase (Barrett & Starkey, 1973; Barrett et al., 1979). The activity of the bound proteinase toward small substrates is retained (Ganrot, 1966, 1967), but activity toward macromolecular substrates is greatly diminished (Harpel & Mosesson, 1973; Bieth et al., 1981; Gonias & Pizzo, 1983a).

Each mole of α_2M is capable of inhibiting 2 mol of trypsin or chymotrypsin (Pochon et al., 1978; Barrett et al., 1979; Swenson & Howard, 1979) but only 1 mol of plasmin (Ganrot, 1967; Pochon et al., 1978; Gonias et al., 1982a) or a synthetic chymotrypsin dimer (Pochon et al., 1981). This difference in binding can be explained by a model of α_2M structure that contains two adjacent and equivalent binding sites (Pochon et al., 1981; Pochon & Bieth, 1982; Feldman et al., 1985). A large proteinase such as plasmin can bind to one site and sterically inhibit the binding of a second proteinase (Pochon et al., 1981; Gonias & Pizzo, 1983a; Feldman et al., 1985).

Limited reduction and alkylation of α_2M results in the generation of two functional "half-molecules", each containing one proteinase binding site (Gonias & Pizzo, 1983a,b). When a proteinase enters a binding site, it cleaves both subunits (Pochon et al., 1981), two thiol groups are generated (Sottrup-Jensen et al., 1980, 1981), and a change in the conformation of the inhibitor occurs exposing two receptor recognition sites (Imber & Pizzo, 1981; Kaplan et al., 1981; Van Leuven et al., 1979). Methylamine can also cause the appearance of these thiol groups and an equivalent conformational change in human α_2M (Gonias et al., 1982; Barrett et al., 1979). This change in conformation can be demonstrated by a "slow" to "fast" shift in electrophoretic mobility in

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nondenaturing polyacrylamide gel electrophoresis (PAGE) (Barrett et al., 1979; Nelles et al., 1980) or by other physicochemical techniques (Barrett et al., 1979; Gonias et al., 1982b; Bjork & Fish, 1982; Strickland & Bhattacharya, 1984). In many non-human α-macroglobulins, however, reaction with CH_3NH_2 is insufficient to induce a slow to fast conformational change (Feldman et al., 1984; Feldman & Pizzo, 1984).

α_2M–proteinase complexes are rapidly cleared from the circulation by hepatocytes and reticuloendothelial cells following the conformational change, that exposes receptor recognition sites on the inhibitor (Ohlsson, 1971; Imber & Pizzo, 1981; Glieman et al., 1983; Feldman et al., 1983). Studies of receptor recognition based on in vivo plasma clearance studies have demonstrated a pattern of clearance of α_2M–plasmin that is different than that of other α_2M–proteinase complexes (Gonias et al., 1982; Gonias & Pizzo, 1983a), but in vitro macrophage binding studies have failed to demonstrate a difference in receptor affinity (Ney et al., 1985). This investigation was undertaken to explain this discrepancy. These studies suggest that the mechanism of subunit cleavage of α_2M by plasmin has an important effect on receptor recognition site exposure.

Experimental Procedures

Materials. The sources for reagents are listed elsewhere (Gonias & Pizzo, 1983b; Ney et al., 1985).

Proteins. Human α_2M was prepared by the method of Kurecki et al. (1979) as modified by Imber and Pizzo (1981). Bovine pancreatic trypsin inhibitor (BPTI) was purchased under the trade name Trasylol from Mobay Chemical Corp., Pittsburgh, PA. Human α-thrombin was prepared as described by Fenton et al. (1977) and possessed an activity of 2700 units/mg and was 95% α-thrombin. Trypsin was obtained by Sigma and dissolved in 1 mM HCl. Plasminogen was purified from human plasma as described by Deutsch and Mertz (1970) and modified by Brockway and Castellino (1972). Plasmin was prepared by incubation of plasminogen with urokinase (250–500 units/mg of plasminogen). The concentration of active plasmin or active trypsin was determined by active site titration with *p*-nitrophenyl *p*-guanidinobenzoate (PNPGB) (Chase & Shaw, 1967). All proteins were dissolved in 20 mM NaH_2PO_4–100 mM NaCl, pH 7.4 (PBS), unless otherwise noted.

Protein Iodination. α_2M, plasminogen, and trypsin were radiolabeled with $Na^{125}I$ by the solid-state lactoperoxidase method of David and Reisfeld (1974). Specific activities between 300 and 1000 cpm/ng were obtained without loss of protein activity.

Protein Concentrations. Protein concentrations were calculated with the following constants: α_2M and α_2M–CH_3NH_2, $A(1\%,1cm) = 8.93$, M_r 718 000 (Hall & Roberts, 1979); plasminogen, $A(1\%,1cm) = 16.8$, M_r 92 000 (Sjoholm et al., 1973); α_2M–Pm, $A(1\%,1cm) \sim 9.0$, $M_r \sim 800\,000$.

Preparation of α_2M–Plasmin Complexes (α_2M–Pm). Unless otherwise noted, α_2M (0.7 μM) was incubated with plasmin (0.7 μM) for 1 h at 37 °C. In some experiments, 5 mM diisopropyl fluorophosphate (DIFP) was added and the incubation was continued for an additional 18 h at 23 °C. The mixture was then subjected to gel filtration chromatography on Ultrogel AcA-22. The binding ratio of plasmin to α_2M under these conditions was between 0.98:1 and 1.05:1 as determined by gel filtration chromatography with ^{125}I-plasmin. The purified α_2M–Pm complex was stored at 4 °C and used within 3 days. In some experiments, complexes of α_2M–Pm were prepared as described above, and the purified material was stored at −20 °C in glycerol for several months. Aliquots were dialyzed against PBS prior to use.

Preparation of α_2M–CH_3NH_2. α_2M (0.7 μM) was incubated with CH_3NH_2 (0.4 M) for 14 h at 23 °C. Unbound CH_3NH_2 was removed by extensive dialysis against PBS. The preparation was in the "fast" form as determined by nondenaturing PAGE.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate (SDS)–PAGE was performed by the tris(hydroxymethyl)aminomethane (Tris)/sulfate system of Neville (1971) and analyzed by densitometry. Samples were incubated with the upper reservoir buffer containing 65 mM dithiothreitol and denatured for 45 min at 37 °C before application to 5% slab gels. Nondenaturing PAGE was performed on 5% gels by the *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES)/imidazole system of McLellan (1980). In most experiments 7–10 μg of protein was applied to each lane. Samples containing plasmin were allowed to react with 90 μM PNPGB prior to denaturation. In gels containing radioactivity, the protein bands were excised, and the radioactivity present was determined in a γ counter. Protein content in each band was determined from known protein specific activities.

Analysis of PAGE. Automatic integrating densitometry was performed on a Gelman ACD-15 densitometer as previously described (Gonias & Pizzo, 1981). Preparation of samples for SDS–PAGE results in heat fragmentation of some 180-kDa α_2M subunits into 120- and 60-kDa species (Harpel et al., 1979). When the percentage of subunits cleaved by a proteinase is calculated it is necessary to correct the data for the amount of uncleaved subunits by taking into account the extent of heat fragmentation (Christensen & Sottrup-Jensen, 1984).

Radioactive bands were excised and counted in a γ counter. Background radioactivity was determined and subtracted from total radioactivity to obtain protein-bound radioactivity. The protein content was determined from known protein specific activities.

Macrophage Binding Studies. Binding studies were performed at 4 °C with fixed monolayers of thioglycolate-stimulated murine peritoneal macrophages (Adams, 1979). The procedure is described in detail elsewhere (Ney et al., 1985). Briefly, cells were allowed to equilibrate at 4 °C in binding buffer. Before the experiment, the buffer was aspirated, and radiolabeled ligand in binding buffer was placed over the cells. Cells were then washed and the cell pellets solubilized and counted in a γ counter. The protein content of the pellet was determined by the method of Lowry et al. (1951) as modified by Peterson (1977).

Plasma Clearance Studies. Clearance studies were performed in 20-week-old CD-1 female white mice. A 400-μL solution of PBS containing ^{125}I-α_2M was injected into the lateral tail vein of the mouse. All samples containing proteinases were incubated with BPTI before injection. Twenty-five microliters of blood was removed at various time intervals from the retroorbital venous plexus and counted for γ radioactivity. The radioactivity in the sample withdrawn within 10 s after injection was defined as the initial ligand concentration, and the radioactivity present in subsequent aliquots was represented as a percentage of this first measurement (Imber & Pizzo, 1981).

Results

Binding of α_2M to Macrophages. The binding of α_2M–CH_3NH_2 or α_2M–Pm to murine peritoneal macrophages was studied at 4 °C (Figure 1). The amount of α_2M–Pm bound per milligram of cell protein, the receptor binding activity, was approximately half that for α_2M–CH_3NH_2 or α_2M–trypsin

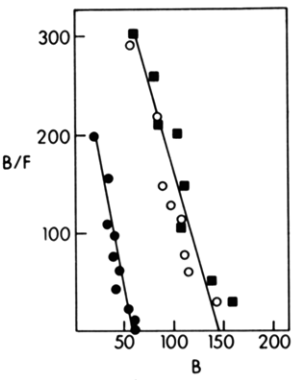

FIGURE 1: Comparison of binding of α_2M–CH_3NH_2 and α_2M–Pm to macrophages. Various concentrations of ^{125}I-α_2M–CH_3NH_2 (■), freshly prepared ^{125}I-α_2M–Pm (●), and ^{125}I-α_2M–Pm that had been stored at −20 °C in glycerol before use (○) were incubated with murine peritoneal macrophages at 4 °C. Nonspecific binding was determined by incubation of labeled ligand in the presence of ethylenediaminetetraacetic acid (EDTA) and subtracted from total binding to determine specific binding. Specific binding (fmol/mg of cell protein) is plotted according to the method of Scatchard (1949). Points shown are the mean of triplicate determinations.

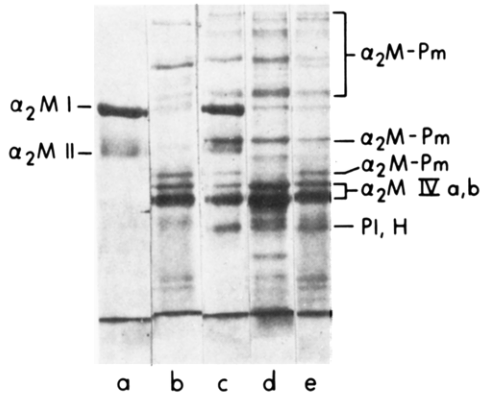

FIGURE 2: SDS–PAGE of α_2M–proteinase complexes. Complexes of α_2M–trypsin or α_2M–Pm were formed by the incubation of a 2-fold molar excess of proteinase with α_2M for 1 h at 37 °C. (Lane a) Native α_2M; (lane b) α_2M–trypsin; (lane c) α_2M–Pm; (lane d) α_2M–Pm incubated with a 4-fold excess of trypsin for 10 min at 23 °C; (lane e) α_2M–Pm that had been stored at −20 °C in glycerol prior to use. The labeled bonds include the following: α_2M I, the M_r ~180 000 α_2M subunit; α_2M II, the M_r ~120 000 heat fragmentation band; α_2M IVa,b, the M_r ~95 000 and 85 000 subunit cleavage products resulting from reaction with proteinase; PI, H, the plasmin heavy chain. The bands labeled α_2M–Pm result from covalent adducts of the plasmin heavy and light chains to cleaved subunits of α_2M as previously described (Gonias et al., 1982a, 1983b).

(60 vs. 140 fmol/mg of cell protein). These complexes, however, bound to the receptor with the same affinity and an apparent K_d of 0.4 nM. A previous study from this laboratory reported identical receptor binding activities for these three ligands (Ney et al., 1985). This earlier study was performed with a preparation of α_2M–Pm that had been stored at −20 °C in glycerol for months. This material and freshly prepared α_2M–Pm were subjected to SDS–PAGE (Figure 2). Densitometry revealed that 96% of the α_2M subunits had been cleaved by plasmin in the stored preparation but only 56% had been cleaved in the freshly prepared material.

Plasma Clearance of α_2M–Pm. The clearance of ^{125}I-α_2M–Pm from the plasma of mice is presented in Figure 3. As has been previously reported, the clearance of purified α_2M–Pm was biphasic with a half-life of 5 min (Gonias et al., 1982). To rule out the possibility that this was due to some contaminating native α_2M, an incubation of ^{125}I-α_2M with a 2-fold molar excess of plasmin was adsorbed to lysine–Sepharose, washed with PBS, and eluted with 0.5 M lysine. As previously reported and confirmed in this study, native α_2M does not adsorb to lysine–Sepharose, and this procedure eliminates all unreacted α_2M from the preparation of α_2M–plasmin (Cummings & Castellino, 1984). The clearance of this preparation of α_2M–Pm was identical with that described above (Gonias et al., 1982). When this material was treated with a 4-fold molar excess of trypsin for 10 min or 0.4 M CH_3NH_2 for 4 h, the complex cleared with a half-life of 2 min. This pattern was indistinguishable from the first-order clearance of α_2M–trypsin or α_2M–CH_3NH_2 (Imber & Pizzo, 1981). SDS–PAGE revealed that the addition of trypsin resulted in the cleavage of all but 5% of the remaining subunits in the α_2M–Pm complex (Figure 2, lane d) and that the addition of CH_3NH_2 did not result in additional subunit cleavage (results not shown).

When α_2M–Pm is subjected to nondenaturing PAGE, it demonstrates a characteristic mobility intermediate between slow and fast forms that are produced by trypsin or CH_3NH_2 treatment (Ney et al., 1985). As demonstrated in Figure 4,

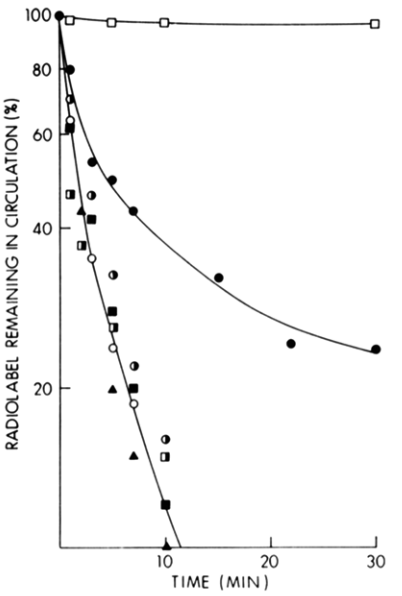

FIGURE 3: In vivo plasma elimination of α_2M–proteinase complexes. In each experiment, 1.0×10^6 cpm of radiolabeled α_2M was injected into the mouse. The studies include the following: α_2M incubated with a 2-fold molar excess of plasmin and purified by affinity chromatography on lysine–Sepharose (●); this material incubated with a 4-fold excess of trypsin (■) or 0.4 M CH_3NH_2 (◨); α_2M incubated with a 20-fold molar excess of plasmin for 1 h at 37 °C (◑); α_2M–Pm that had been stored at −20 °C in glycerol (▲). The clearances of native α_2M (□) and α_2M–trypsin (○) are shown for comparison.

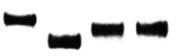

FIGURE 4: Nondenaturing PAGE of α_2M–proteinase complexes. Native α_2M (lane a), α_2M–trypsin (lane b), α_2M–Pm (lane c), and trypsin-treated α_2M–Pm (lane d) were subjected to nondenaturing PAGE as described under Experimental Procedures.

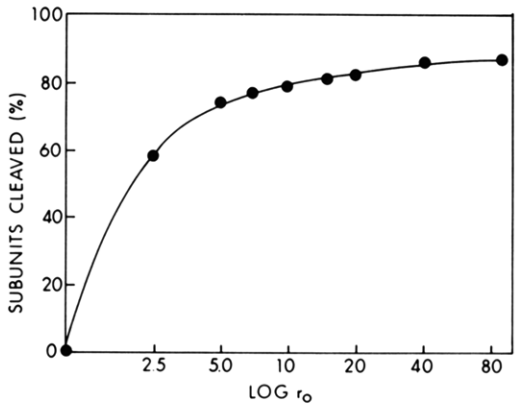

FIGURE 5: Subunit cleavage of α_2M by plasmin at various molar ratios of proteinase to inhibitor (r_0). ^{125}I-α_2M was incubated with plasmin at various molar ratios for 1 h at 23 °C and was analyzed by SDS–PAGE and densitometry. The amount of subunits cleaved by plasmin was plotted as a function of r_0.

tryspin treatment of α_2M–Pm did not alter the mobility pattern of α_2M–Pm.

The preparation of α_2M–Pm that behaved equivalently to that of α_2M–trypsin or α_2M–CH_3NH_2 in vitro was radiolabeled and injected into the mouse. This material cleared in a rapid, first-order pattern like α_2M–trypsin of α_2M–CH_3NH_2 (Figure 3). SDS–PAGE revealed that all but 4% of the subunits of α_2M had been cleaved in this preparation of α_2M–Pm (Figure 2, lane e).

^{125}I-α_2M was incubated with a 20-fold molar excess of plasmin for 1 h at 37 °C. When this material was injected into the mouse, it cleared rapidly in a first-order reaction like α_2M–trypsin (Figure 3). SDS–PAGE of this material revealed that 85% of the subunits of α_2M had been cleaved in this preparation of α_2M–Pm (see Figure 6, lane e).

Experiments were performed to explore the possibility that the biphasic clearance of α_2M–Pm is due to the interaction of α_2M-bound plasmin with some factor in mouse plasma. If this hypothesis were correct, the effect of trypsin on the clearance of α_2M–Pm (Figure 3) might result from cleavage of the plasmin heavy chains that protrude from the α_2M binding site (Cummings & Castellino, 1984). A purified complex of α_2M reacted with ^{125}I-plasmin was treated with a 4-fold molar excess of trypsin for 30 min. The material was then subjected to gel filtration chromatography on Sephacryl S-300. Ninety-four percent of the radioactivity remained bound to the α_2M–Pm complex. It is highly unlikely, therefore, that the altered clearance of trypsin-treated α_2M–Pm is due to proteolysis of plasmin bound to α_2M.

Subunit Cleavage of α_2M by Plasmin. Plasmin was incubated with ^{125}I-α_2M at various molar ratios for 1 h at 37 °C. As Figure 5 shows, the degree of subunit cleavage was dependent upon the concentration of plasmin present in the incubation. A 2.5-fold molar excess cleaved 58% of the subunits, whereas a 40-fold molar excess of plasmin cleaved 86% of the subunits under identical incubation conditions.

To determine whether the additional subunit cleavage was due to a second proteinase binding to α_2M the following experiment was performed. A 20-fold molar excess of ^{125}I-plasmin was incubated with α_2M for 24 h at 37 °C and the complex subjected to gel filtration chromatography on Sephacryl S-300. The binding ratio of plasmin to α_2M was 0.98:1 as determined by the incorporation of ^{125}I-plasmin. In this preparation of α_2M–Pm, greater than 90% of the subunits of α_2M had been cleaved as determined by SDS–PAGE. These results strongly suggest that the additional cleavage is not due to a second bound proteinase. This finding prompted us to explore the mechanism of α_2M subunit cleavage by plasmin.

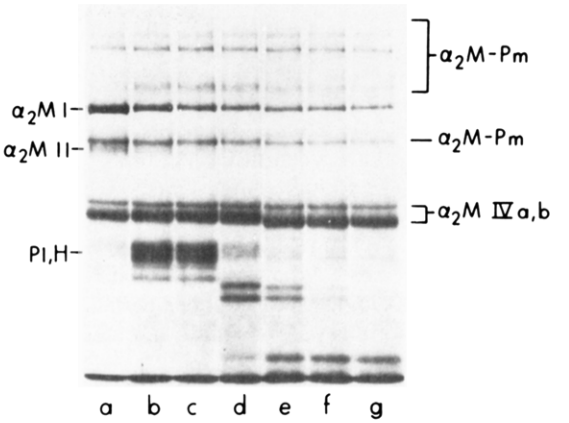

FIGURE 6: SDS–PAGE of α_2M–Pm. DIFP-treated α_2M–Pm was purified as described under Experimental Procedures. This material was incubated with a 4-fold molar excess of plasmin at 23 °C. Aliquots were removed at various times and subjected to SDS–PAGE. The time of incubation was (a) 0, (b) 1 h, (c) 9 h, (d) 1 day, (e) 3 days, (f) 5 days, and (g) 8 days. The protein bands are designated as in Figure 2. The bands below the plasmin heavy chain, Pl, H, are degradation products of the heavy chain as demonstrated by autoradiography of the gel employing ^{125}I-plasmin.

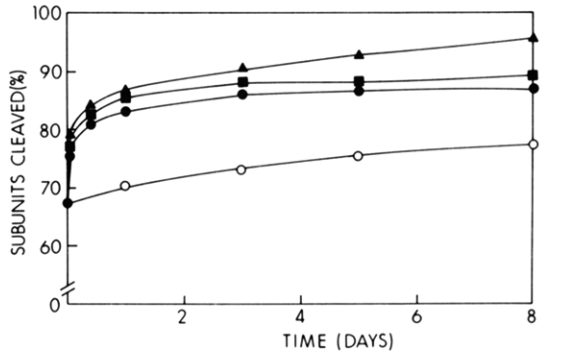

FIGURE 7: Subunit cleavage of α_2M by bound or unbound plasmin. A gel such as that shown in Figure 6 was analyzed by densitometry and excision and counting of the radioactive bands. The percentage of DIFP-treated α_2M–Pm subunits cleaved by a 4-fold molar excess of plasmin at 4 (●), 23 (■), of 37 °C (▲) is plotted as a function of time of incubation. Purified α_2M–Pm was incubated alone at 37 °C (○) and subjected to identical analysis. The values are the average of duplicate determinations.

The amount of subunit cleavage of α_2M by plasmin bound or unbound to α_2M was studied by SDS–PAGE. In one set of experiments, ^{125}I-α_2M–Pm was reacted with 5 mM DIFP and the reaction mixture subjected to gel fitration chromatography. Sixty-six percent of the α_2M subunits were cleaved in this preparation of α_2M–Pm, as determined by SDS–PAGE and densitometry. Samples of the purified α_2M–Pm complex were subsequently incubated with a 4-fold molar excess of plasmin at 4, 23, or 37 °C. Aliquots containing 10 μg of α_2M–Pm were removed at various times and analyzed by SDS–PAGE and densitometry as shown in Figure 6.

Figure 7 shows the time dependence of the reaction between plasmin and α_2M–Pm complex at various temperatures. The amount of time required to cleave half of the remaining subunits of α_2M–Pm by added plasmin is designated the $t_{1/2}$ for the reaction. The $t_{1/2}$ for the incubations at 4, 23, and 37 °C were 28, 12, and 9 h, respectively. Continued subunit cleavage continued for at least 10 days. Control experiments were performed to confirm that added proteinase did not become incorporated into the α_2M–Pm complex (Gonias & Pizzo, 1983b). The phenomenon of additional subunit cleavage by proteinases without incorporation of additional proteinase

is designated "nonproductive" cleavage.

In another set of experiments, DIFP was omitted after the reaction of ^{125}I-α_2M with plasmin. The purified α_2M–Pm complex was then incubated at 37 °C. Aliquots containing 10 μg of α_2M–Pm were removed at various times and analyzed by SDS–PAGE. This preparation had 66% of the subunits cleaved initially as determined by SDS–PAGE and densitometry. Figure 7 shows that further cleavage of α_2M–Pm subunits by bound plasma did occur slowly. After 2 weeks at 37 °C, only one-third of the remaining subunits were cleaved by this bound plasmin, whereas under the same conditions additional plasmin cleaved essentially all of the remaining subunits.

Subunit Cleavage of α_2M–Pm or α_2M–CH_3NH_2 by Various Proteases. The extent of nonproductive subunit cleavage of α_2M–Pm or α_2M–CH_3NH_2 by various proteinases was determined by SDS–PAGE. Purified α_2M–Pm or α_2M–CH_3NH_2 was reacted with a 4-fold molar excess of either trypsin, α-thrombin, or plasmin for 30 min at 23 °C and subjected to SDS–PAGE. The gel was then analyzed by densitometry. There was 100% nonproductive subunit cleavage of α_2M–Pm by trypsin, 76% by α-thrombin, and only 19% by plasmin. By comparison, the extent of nonproductive subunit cleavage of α_2M–CH_3NH_2 was 23% by trypsin, and there was essentially no nonproductive cleavage by α-thrombin or plasmin.

Discussion

Studies of α_2M–Pm have resulted in several inconsistent observations. These discrepancies are of two types: namely, the extent of α_2M subunit cleavage observed when plasmin binds to the inhibitor and the nature of the interactions of α_2M–Pm and cell surface receptors. In this study, when α_2M was incubated with various molar ratios of plasmin, the amount of subunit cleavage was dependent upon the concentration of plasmin. At a molar ratio of 2.5, about half of the subunits of α_2M had been cleaved. Increasing the plasmin to α_2M molar ratio resulted in additional subunit cleavage. At a plasmin to α_2M molar ratio of 20, 86% of the subunits of α_2M had been cleaved, but only 1 mol of plasmin was bound per mole of α_2M. This result implies that additional subunits of α_2M are cleaved either by plasmin bound to α_2M or by plasmin that cannot bind to α_2M. Each of these possible mechanisms was examined in detail.

When purified complexes of DIFP-treated α_2M–Pm were incubated with excess plasmin, the proteinase cleaved the remaining subunits of α_2M–Pm and did not become incorporated into the complex. This additional or nonproductive cleavage was dependent upon length of incubation and on temperature. In addition to nonproductive subunit cleavage by unbound plasmin, the bound plasmin can continue to cleave subunits of α_2M–Pm, but at a greatly reduced rate. These findings and the effect of plasmin concentration on α_2M subunit cleavage probably explain the varying extent of subunit cleavage of α_2M by plasmin reported by different investigators.

Studies of cell binding and plasma clearance of α_2M–Pm complexes revealed a correlation between receptor recognition site exposure and degree of subunit cleavage of α_2M–Pm. Purified α_2M–Pm with half of the subunits of the α_2M cleaved demonstrated about half the in vitro receptor binding of α_2M–trypsin. The receptor binding of α_2M–Pm that had all four subunits of the α_2M cleaved was identical with that of α_2M–trypsin or α_2M–CH_3NH_2. These results can be explained in terms of the extent of receptor recognition site exposure of α_2M. Treatment with CH_3NH_2 or subunit cleavage by trypsin leads to the activation and cleavage of the four thiol ester bonds of α_2M (Sottrup-Jensen et al., 1980, 1981). All four receptor recognition sites are exposed in these α_2M complexes (Marynen et al., 1983; Van Leaven et al., 1983). In a preparation of α_2M–Pm in which only two subunits of α_2M are cleaved, only two receptor recognition sites are exposed, and it would be reasonable to predict that the in vitro receptor binding would be significantly less than that of a preparation of α_2M–trypsin. The binding studies presented here demonstrate that the cleavage of the remaining subunits of α_2M–Pm by plasmin results in complete receptor recognition site exposure and a 2-fold increase in receptor binding. The in vivo clearance studies of receptor recognition are also consistent with the observation that the receptor binding of the complex is increased by additional subunit proteolysis or by treatment with CH_3NH_2. These observations confirmed and extended our previous studies with bovine α_2M that changes in conformation as determined by nondenaturing PAGE may not correlate with changes in receptor binding (Feldman et al., 1984).

In conclusion, it has been shown that treatment of α_2M with plasmin results in proteolysis of half of the subunits of α_2M leading to half-maximal receptor recognition site exposure. Additional thiol ester bond cleavage either by subunit proteolysis or CH_3NH_2 activation results in the exposure of additional receptor recognition sites and an increase in receptor binding of α_2M–Pm complexes. Finally, proteolysis of intact subunits of α_2M–Pm complex by plasmin results from nonproductive cleavage by unbound plasmin while bound plasmin cleaves α_2M subunits only at a very slow rate.

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