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Symmetry of the Inhibitory Unit of Human α_2-Macroglobulin†

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ABSTRACT: Human α_2-macroglobulin (α_2M) of M_r ~720000 is a proteinase inhibitor whose four identical subunits are arranged to form two adjacent inhibitory units. At present, the spatial arrangement of the two subunits which form one inhibitory unit (the functional "half-molecule") is not known. Treatment of α_2M with either 0.5 mM dithiothreitol (DTT) or 4 M urea results in dissociation of the native tetramer into two half-molecules of M_r ~360000. These half-molecules retain trypsin inhibitory activity, but in each case, the reaction results in reassociation of the half-molecules to produce tetramers of M_r ~720000. However, when reacted with plasmin, the preparations of half-molecules have different properties. DTT-induced half-molecules protect the activity of plasmin from inhibition by soybean trypsin inhibitor (STI) without reassociation, while urea-induced half-molecules show no ability to protect plasmin from reaction with STI. High-performance size-exclusion chromatography and sedimentation velocity ultracentrifugation studies were then used to estimate the Stokes radius (R_e) of α_2M and both DTT- and urea-induced half-molecules of α_2M. The R_e of tetrameric α_2M was 88-94 Å, while that of DTT-induced half-molecules was 57-60 Å and urea-induced half-molecules 75-77 Å. These results demonstrate that DTT- and urea-induced half-molecules have fundamentally different molecular dimensions as well as inhibitory properties. The hydrodynamic data suggest that the urea-induced half-molecule is a "rod"-like structure, although it is not possible to predict the three-dimensional structure of this molecule with the available data. The data are most consistent with the conclusion that the DTT-induced half-molecule is a "basket"-like structure, and this half-molecule is the basic inhibitory unit of α_2M.

The α-macroglobulins are a group of high molecular weight proteins that bind and inhibit a diverse variety of proteinases including members from all four catalytic classes [for reviews, see Roberts (1986) and Sottrup-Jensen (1987)]. Their inhibitory capacity results from a conformational change following proteinase reaction that appears to physically entrap the reacting proteinase molecule(s) (Barrett & Starkey, 1973), and they are therefore very different from the other, active-site-directed protein inhibitors of proteinases (Travis & Salvesen, 1983).

Human α_2-macroglobulin (α_2M) is the most intensively studied α-macroglobulin. α_2M is a tetrameric molecule formed by the noncovalent association of two disulfide-bonded subunits (Harpel, 1973). The primary structure of each M_r ~180000

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subunit is identical, and the four subunits are arranged to form two adjacent proteinase binding sites (Pochon & Bieth, 1982; Steiner et al., 1985). This architecture is seen in the α_2M homologues found in other mammals and in the protein ovomacroglobulin from avian eggs (Nishigai et al., 1985; Ruben et al., 1988). The α-macroglobulin homologues of plaice (Starkey & Barrett, 1982), the Southern grass frog (Feldman & Pizzo, 1983), and lobster (Spycher et al., 1987), however, are about half the size of human α_2M. Thus, an α-macroglobulin inhibitory unit is composed of a structure containing only two subunits.

α_2M is dissociated into disulfide-bonded "half-molecules" by treatment of the native protein with either 4 M urea (Jones et al., 1973; McConnell & Loeb, 1974; Barrett et al., 1979) or acid (Jones et al., 1973; Barrett et al., 1979). In addition, half-molecules of human α_2M are generated by limited reduction of disulfide bonds with dithiothreitol (DTT) (Gonias & Pizzo, 1983a,b; Sjöberg et al., 1985). DTT-induced half-molecules are stabilized by the noncovalent association of two α_2M subunits, although it is not known whether the half-molecules were covalently or noncovalently associated prior to limited reduction. These half-molecules retain the ability to bind proteinases in a near-equimolar ratio, although their capacity to inhibit the bound proteinase is not as great as for the native tetrameric molecule. As a result of these and other physiochemical data, a model of α_2M structure has been proposed which attempts to explain the apparent symmetry of the two adjacent inhibitory units. In this model, the two half-molecules which form native α_2M are arranged as "back-to-back" proteinase binding sites. The structure of the proteinase binding site of α_2M has been described as resembling one of two "traps" by Feldman et al. (1985), "baskets" by Sottrup-Jensen (1987), and "royal crowns" by Larsson et al. (1987).

Many of the unusual characteristics of the inhibition of proteinases by α_2M have been elucidated, but a consensus of the structure of the native molecule and its basic inhibitory unit (the functional half-molecule) has proven elusive. Recently, Delain et al. (1988) employed Cd^{2+} to obtain a preparation of α_2M for electron microscopy. This preparation contained a small amount of half-molecules, although it has been previously reported that this treatment resulted primarily in the generation of dimers arranged irregularly in polymeric structures of very high molecular weight (Couture-Tosi et al., 1986). Ruben et al. (1988), in their electron microscopy study of ovomacroglobulin, observed half-molecules at a pH below 4. Since Cd^{2+}-induced half-molecules have little proteinase binding capacity (Couture-Tosi et al., 1986) and acid treatment is known to inactivate human α_2M (Barrett et al., 1979), however, these electron microscopy studies say little about the structure of the basic inhibitory unit of α_2M. A recent publication (Liu et al., 1987) has suggested that the functional half-molecule of α_2M can be composed of either two covalently or two noncovalently associated subunits which resemble slender "rod"-like structures. In this model, the half-molecules are arranged "side-to-side" to form the intact tetramer. This hypothesis is in clear conflict with the model of subunit symmetry proposed by Feldman et al. (1985). In this report, we attempt to rationalize these inconsistencies by examining the symmetry and dimensions of half-molecules of α_2M formed by different forms of dissociation, and by analyzing the reaction of α_2M with proteinases under conditions which result in dissociation of the native molecule.

Experimental Procedures

Proteins and Reagents. α_2M was purified as described by Imber and Pizzo (1981). α_2M and bovine trypsin were radiolabeled with $Na^{125}I$ by the method of David and Reisfeld (1974) without substantial loss of protein activity. Plasminogen was purified by affinity chromatography on Sepharose–lysine (Deutsch & Mertz, 1970) and was activated with 500 Plough units of urokinase (Calbiochem) per milligram of plasminogen for 30 min at room temperature. The chromogenic substrates *N*-benzoyl-DL-arginine-*p*-nitroanilide-HCl (BAPNA) and H-D-Val-Leu-Lys-*p*-nitroanilide-HCl (S-2251) were obtained from Sigma and The Kabi Co., respectively. Analytical-grade urea was obtained from Mallinckrodt.

Preparation of Urea-Induced Half-Molecules of α_2M. Samples of α_2M or α_2M–trypsin complex (1 μM) were treated with urea to a final concentration of 4 M for 30 min at room temperature. Urea was dissolved in 90 mM tris(hydroxymethyl)aminomethane hydrochloride, 90 mM boric acid, and 2 mM EDTA, pH 8.6 (TBE buffer), immediately prior to each experiment. All urea-induced half-molecule preparations were formed in the presence of TBE buffer and used immediately. The half-molecule preparations were active as determined by the ability of the preparation to bind ~0.6 mol of ^{125}I-trypsin per mole prior to gel filtration chromatography in 3 M urea.

Preparation of DTT-Induced Half-Molecules of α_2M. α_2M (1 μM) was treated with 0.5 mM DTT in 120 mM NaP_i, pH 7.4, for 1 h at room temperature. Iodoacetamide (1.3 mM) was then added to alkylate the liberated thiol groups, and excess reagents were removed by exhaustive dialysis against 25 mM HEPES and 150 mM NaCl, pH 7.4, in the dark. Greater than 95% of the intersubunit disulfide bonds in α_2M had been cleaved in the preparation as determined by SDS–PAGE performed under nonreducing conditions. The molecular weight of the preparation was approximately 340 000 as determined by sedimentation equilibrium ultracentrifugation.

High-Performance Size-Exclusion Chromatography. High-performance size-exclusion liquid chromatography was performed by using the LKB system described previously (Gonias et al., 1986). The same TSK G-4000 SW gel filtration chromatography column (7.5 × 300 mm) and TSK-SWP guard column (7.5 × 75 mm) was used in all experiments. For each experiment, the buffer (20 mM NaP_i and 100 mM NaCl, pH 7.2) was filtered through a 0.22-μm filter, and all samples were subjected to centrifugation in an Eppendorf microcentrifuge prior to injection. Samples normally contained 75 μg of protein injected in a volume of 100 μL. The flow rate was maintained at 0.5 mL/min, and the protein absorbance was monitored at 280 nm. The distribution coefficient (K_d) of each protein was calculated by using the equation $K_d = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of the sample. The column void volume (V_0) and the total accessible volume (V_t) were determined by using Blue Dextran 2000 and NaN_3, respectively (Himmel & Squire, 1981). The Stokes radii (R_e) of all column calibration standards used in these studies have been reported previously by Horiike et al. (1983) and the references contained therein.

Previous studies from this laboratory have indicated that nonideal behavior may be observed with TSK G-4000 SW gel filtration columns (Gonias et al., 1986). This behavior is characterized by increased interaction of the protein with the matrix and prolonged retention times with marked peak broadening. On the other hand, Nishigai et al. (1985) did not observe this behavior when α_2M was chromatographed on these columns. Prior to the use of the TSK 4000 SW column, this behavior was examined in greater detail. As noted previously by other investigators (Wilf et al., 1986; P. A. Roche

and C. S. Greenberg, unpublished data), older columns characteristically exhibited decreased flow rates, prolonged retention times, and peak broadening. This behavior was not observed with new columns. In the present study, the column employed was new, and the peaks obtained were sharp and discrete. Moreover, as indicated above, all studies were performed on the same column.

Electrophoresis. Nondenaturing polyacrylamide gel electrophoresis (PAGE) of ^{125}I-α_2M was performed in the TBE buffer described above in linear gradient gels (4–12% T, 3% C). The gradients were formed in the presence of the indicated concentration of urea and electrophoresed overnight at 100 V with buffer recirculation. In some experiments, the urea-induced half-molecule preparations were treated with a 10-fold molar excess of either trypsin or plasmin for 3 min at room temperature. The proteinase was then inactivated with a 4-fold molar excess of bovine pancreatic trypsin inhibitor. Protein bands containing ^{125}I-α_2M were visualized by autoradiography using Kodak XAR film and reagents following the manufacturer's recommendations.

Analytical Ultracentrifugation. Sedimentation velocity ultracentrifugation was performed as previously described (Gonias et al., 1982) in a Spinco Model E analytical ultracentrifuge in a buffer of 20 mM NaP_i and 100 mM NaCl, pH 7.4. All protein samples were studied in the same buffer. The Stokes radii of the protein preparations were calculated on the basis of the parameters for native α_2M and α_2M half-molecules (Gonias et al., 1982; Gonias & Pizzo, 1983a). The Stokes radius of urea-treated α_2M was estimated indirectly as a function of the molecular weight and the sedimentation coefficient of the preparation as previously described (Pochon et al., 1978).

Proteinase Activity Measurements. The activity of trypsin or plasmin following reaction with α_2M in various concentrations of urea was assayed by a modification of the methods of Ganrot (1966) for trypsin and Cummings and Castellino (1984) for plasmin. Trypsin or plasmin (0.5 μM) was incubated alone or with a 2-fold molar excess of either native α_2M or urea-induced half-molecules of α_2M in the appropriate concentration of urea/TBE buffer for 3 min at room temperature. A 4-fold molar excess of soybean trypsin inhibitor (STI) was then added, and 5 min later, the proteinase activity was determined by using the chromogenic substrate BAPNA for trypsin and S-2251 for plasmin. The final concentration of proteinase during these assays varied from 10 to 25 μg/mL. In a related series of experiments, proteinase active-site concentration was determined by using the titrant *p*-nitrophenyl *p*-guanidinobenzoate (NPGB) (Chase & Shaw, 1967). A constant concentration of urea was maintained in all experiments. The activity of either proteinase in urea was expressed as a percentage of the activity in the absence of urea.

RESULTS

Physicochemical Studies of α_2M. Among the possible models of the symmetry of the basic inhibitory unit of native α_2M, the two most likely models are ones in which two of the α_2M subunits (the half-molecules) are bounded by a plane parallel to either (a) the short axis or (b) the long axis of the native tetramer (see Figure 1). In an attempt to distinguish which symmetry is correct, experiments were performed to determine the Stokes radius (R_e) of half-molecules formed by dissociation of α_2M in 0.5 mM DTT (DTT-induced half-molecules) and half-molecules formed by dissociation of α_2M in 4 M urea (urea-induced half-molecules). High-performance size-exclusion chromatography and sedimentation velocity ultracentrifugation were used to determine R_e. The data from these studies are shown in Figure 2 and Table I. The R_e for native α_2M is 94 Å as determined in the ultracentrifuge and 88 Å by high-performance size-exclusion chromatography. Reaction with proteinase or methylamine results in compacting

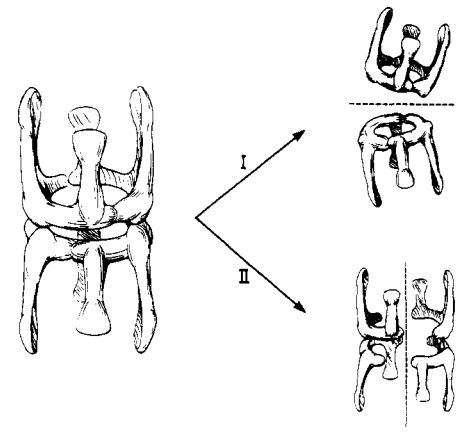

FIGURE 1: Model of α_2-macroglobulin and two possible modes of dissociation. On the left of the figure is a representation of the structure and subunit symmetry of α_2M as proposed by Feldman et al. (1985). The overall shape of this molecule is essentially the same as that proposed by others (Bloth et al., 1968; Barrett & Starkey, 1974; Österberg & Malmensten, 1984; Liu et al., 1987; Ruben et al., 1988). The products of two possible modes of dissociation of the native molecule are represented by pathways I and II.

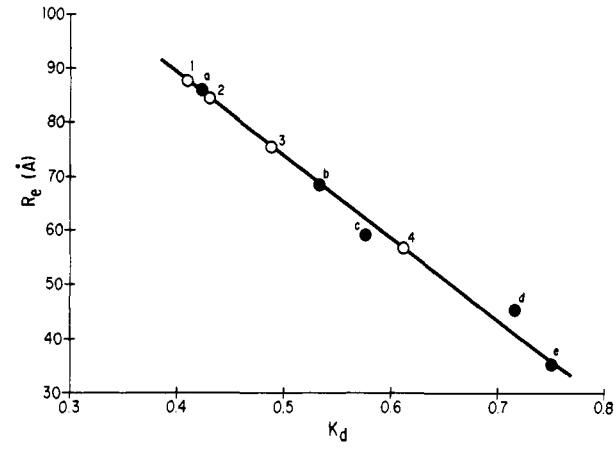

FIGURE 2: Determination of the Stokes radius of α_2M or α_2M half-molecules. Native α_2M (1), α_2M–trypsin complex (2), urea-induced half-molecules of α_2M (3), and DTT-induced half-molecules of α_2M (4) were applied to a TSK G4000 SW gel filtration column. The K_d of each protein was determined as described in the text and was plotted on the calibration curve shown above. The R_e values of the protein standards are as follows: (a) thyroglobulin (86 Å); (b) β-galactosidase (69 Å); (c) ferritin (59 Å) (d) aldolase (46 Å); and (e) albumin (35 Å).

Table I: Stokes Radii (Å) of α_2M and Half-Molecule Preparations

protein	technique employed: ultracentrifuge	technique employed: chromatography	model
α_2M	94[a,b,c,d]	88[d]	90[e]
α_2M–trypsin	91[c]	88[d,f]	87[e]
DTT-induced α_2M half-molecule	60[d]	57[d]	58[e]
urea-induced α_2M half-molecules	77[a,b]	75[d]	

[a] Data from Jones et al. (1973). [b] Data from McConnell and Loeb (1974). [c] Data from Gonias et al. (1982). [d] Data from present study. [e] Calculations based on the α_2M model previously presented in Feldman et al. (1985). No prediction can be made a priori for the urea-induced half-molecule. The possible locations of the disulfide bonds are discussed in the text. [f] Data from Nishigai et al. (1985).

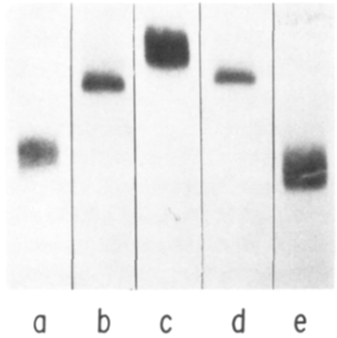

FIGURE 3: Nondenaturing PAGE of urea-induced half-molecules of α_2M. Urea-induced half-molecules were prepared by incubation of α_2M (1 μM) with 4 M urea in TBE buffer for 30 min at room temperature. The radiolabeled samples subjected to 4-12% gradient PAGE in the presence of 4 M urea were urea-induced half-molecules (a), preformed α_2M–trypsin complex (b), preformed α_2M–plasmin complex (c), and urea-induced half-molecules following treatment with either a 10-fold molar excess of trypsin (d) or plasmin (e). The electrophoretic mobility of α_2M in polyacrylamide gels not containing urea was slightly less than that of the α_2M–plasmin complex shown in lane c. The slower electrophoretic mobility of the preformed α_2M–plasmin complex relative to the preformed α_2M–trypsin complex has been described previously (Roche & Pizzo, 1987).

of the molecule. The results obtained in the present study are in good agreement with previous measurements using both sedimentation velocity ultracentrifugation (Gonias et al., 1982; Björk & Fish, 1982) and gel filtration chromatography (Nishigai et al., 1985). By contrast, DTT-induced half-molecules eluted at a position corresponding to an R_e of 57 Å when studied by high-performance size-exclusion chromatography. This is in excellent agreement with an R_e of 60 Å for DTT-induced half-molecules as determined by sedimentation velocity ultracentrifugation.

Initial attempts to determine the R_e of urea-induced half-molecules consisted of high-performance size-exclusion chromatography performed in PBS buffer containing 3 M urea; however, all preparations eluted at a position near the void volume of the column. This is most likely due to a decrease of the effective pore size of the matrix in urea, since under these conditions approximately 20% of the total accessible volume of the pores is occupied by urea. For this reason, high-performance size-exclusion chromatography of urea-induced half-molecules in the nondenaturing buffer system described under Experimental Procedures was performed. The half-molecule preparation eluted as a single symmetrical peak in this buffer, and the R_e of urea-induced half-molecules in this buffer was estimated at 75 Å, intermediate between that of tetrameric α_2M and DTT-induced half-molecules. This value is in excellent agreement with the value of 77 Å obtained by two independent laboratories in the analytical ultracentrifuge (Table I).

Reassociation of α_2M Half-Molecules. Treatment of α_2M with 4 M urea resulted in dissociation of the noncovalently associated α_2M subunits and formation of M_r ~360 000 half-molecules of α_2M (Figure 3). Pretreatment of α_2M with either trypsin or plasmin prevented its dissociation in 4 M urea, results which are consistent with those reported by Barrett et al. (1979). Figure 3 also demonstrates that whereas addition of excess trypsin to urea-induced half-molecules resulted in near complete reassociation of the half-molecules, addition of excess plasmin did not. As mentioned above, the addition of trypsin to urea-induced half-molecules resulted in the binding of ~0.6 mol of trypsin/mol of α_2M. It is thus clear that binding of trypsin to urea-induced half-molecules results in the reassociation of two half-molecules.

Table II: Activity of α_2M-Bound Proteinase following Treatment with STI

protein	proteinase activity (%)[b]	
	trypsin	plasmin
α_2M[a]	90	75
DTT-treated α_2M	40	50
urea-treated α_2M[a]	80	7

[a] Native α_2M was incubated in TBE buffer alone (tetrameric α_2M) or 4 M urea (urea-induced half-molecules) prior to incubation with trypsin or plasmin as described under Experimental Procedures. The proteinase activity was then measured with nitroanilide substrates following the addition of a 4-fold molar excess of STI. α_2M had a minimal effect (<10%) on the inhibition of proteinase activity in the absence of STI. [b] The activity of α_2M-bound proteinase following treatment with STI is expressed as a percentage of the activity in TBE buffer alone (for tetrameric α_2M) or 4 M urea (for urea-induced half-molecules of α_2M) in the absence of α_2M and STI.

Inhibition of Trypsin and Plasmin. The activity of trypsin or plasmin in 4 M urea/TBE buffer was compared to that in TBE buffer alone as described under Experimental Procedures. Both proteinases showed a first-order decay in activity when incubated in 4 M urea. The rate constant for the inactivation of either trypsin or plasmin in 4 M urea was approximately 0.3×10^{-3} s^{-1} when measured with the active-site titrant NPGB, while this constant was approximately 0.3 s^{-1} when measured with the nitroanilide substrates. These results may reflect a pronounced effect on deacylation rates in the presence of 4 M urea. This decay in activity was controlled for by incubating all reactants in 4 M urea under identical conditions.

The extent of inhibition of trypsin or plasmin bound to α_2M in 4 M urea/TBE buffer was determined, and the results of the inhibition assays are shown in Table II. Although both trypsin and plasmin had lost a considerable amount of their activities against the nitroanilide substrates in 4 M urea/TBE buffer, it is clear that the residual activity of α_2M-bound trypsin was substantially protected from inhibition by STI. This is in marked contrast to the results obtained with plasmin, in which urea-induced half-molecules protected only 7% of the residual plasmin activity from inhibition by STI (Table II). Data for DTT-induced half-molecules are also included in Table II. These data are consistent with our previous report (Gonias & Pizzo, 1983a,b). It should be noted that the ability of DTT-induced half-molecules to protect trypsin from inhibition by STI is about 40% that of native α_2M, although the extent of covalent binding of trypsin is comparable to that of native α_2M (Gonias & Pizzo, 1983a). This difference results from the fact that STI is able to bind to the half-molecule–trypsin preparation (Gonias & Pizzo, 1983a).

DISCUSSION

Depending on the conditions employed, treatment of α_2M with a number of disulfide-reducing agents results in dissociation of the native tetramer into M_r ~180 000 "quarter-molecules" (Barrett et al., 1979; Larsson et al., 1988) or M_r ~360 000 half-molecules (Gonias & Pizzo, 1983a,b; Sjöberg et al., 1985). Treatment of α_2M with 3–4 M urea results exclusively in dissociation of α_2M into M_r ~360 000 half-molecules (Jones et al., 1972; McConnell & Loeb, 1974; Barrett et al., 1979; Liu et al., 1987). Subsequent reaction of any of these forms with trypsin can result in reassociation to the tetrameric form with concomitant inhibition of the proteinase. This is almost certainly due to an increase in the strength and/or number of subunit interactions created during the conformational changes brought about by proteinase binding by α_2M as discussed by Barrett et al. (1979). The conclusion of Liu et al. (1987) that complexes of urea-induced

half-molecules with trypsin "were observed to migrate as half-molecules" in gel electrophoresis cannot be reconciled with the data from this and other investigations.

In contrast to the results obtained with the proteinase trypsin, reaction of either DTT- or urea-induced half-molecules with the large proteinase plasmin does not result in half-molecule reassociation. It has been proposed that the failure of DTT-induced half-molecules to reassociate following treatment with plasmin is due to protrusion of plasmin across the "contact area" between the two half-molecules (Gonias & Pizzo, 1983a,b). The most striking feature of these two types of half-molecules, however, is their different abilities to inhibit the activity of the proteinase plasmin. Urea-induced half-molecules, unlike DTT-induced half-molecules, are unable to inhibit plasmin. This observation conclusively demonstrates that the half-molecules produced by DTT and urea have very different properties. In fact, taken together with the results of trypsin inhibition assays, it is apparent that inhibition of proteinase activity by urea-induced half-molecules results solely from reassociation of two half-molecules and is not an inherent property of the half-molecule. The ability of DTT-induced half-molecules to inhibit plasmin without undergoing half-molecule reassociation suggests that the DTT-induced half-molecule is the basic inhibitory unit of α_2M.

It is interesting to note that there are differences in the ability of each half-molecule preparation to protect trypsin from inhibition by STI. The urea-induced half-molecules undergo virtually complete reassociation when treated with trypsin, and the trypsin is almost as well protected from reaction with STI as trypsin bound to untreated α_2M. DTT-induced half-molecules also reassociate when treated with trypsin, but either prior to or during reassociation of the half-molecules, the α_2M-bound trypsin reacts with STI (Gonias & Pizzo, 1983a). These results may reflect differences in the products of proteinase-induced reassociation of DTT- and urea-induced half-molecules.

Figure 1 shows the model of α_2M structure proposed by Feldman et al. (1985) and representations of two modes of α_2M subunit symmetry derived from this model. On the basis of the molecular dimensions of this model, the R_e of the basketlike half-molecule was estimated as 58 Å. This value is in excellent aggreement with the R_e of DTT-induced half-molecules determined by both high-performance size-exclusion chromatography and sedimentation velocity ultracentrifugation obtained in this investigation. These results are also consistent with the results obtained by using another estimate of the effective size of the molecule, the radius of gyration, as determined by both low-angle X-ray and neutron-scattering studies (Sjöberg et al., 1985). By contrast, the R_e of the urea-induced half-molecule as determined by high-performance size-exclusion chromatography was 75 Å. This value is in agreement with an R_e of 77 Å for urea-treated α_2M which we have calculated from previously reported sedimentation velocity ultracentrifugation data (Jones et al., 1973; McConnell & Loeb, 1974). It is concluded, therefore, that while treatment of native α_2M with either DTT or urea results in the formation of half-molecules of α_2M, the molecular dimensions of these half-molecules are very different. This conclusion is in disagreement with with that of Liu et al. (1987), who suggested that DTT- and urea-induced half-molecules have nearly identical symmetries and molecular dimensions.

Although DTT-induced half-molecules are formed by cleavage of the interchain disulfide bonds in human α_2M, the relationship of these bonds to the stabilization of the basic inhibitory unit and the tetrameric protein is not known. The location of these bonds in the primary structure of α_2M has been determined (Jensen & Sottrup-Jensen, 1986), but this does not clarify any of the proposed models of α_2M structure. There are two possibilities for the location of the interchain disulfide bonds in relation to the structure of the tetrameric inhibitor, and each one is supported by experimental data. The first model is one in which these bonds are located at the interface of the two basketlike inhibitory units. This requires that the two subunits of the inhibitory unit are associated noncovalently, as is the case found in the M_r ~36 000 α-macroglobulin of the Southern grass frog and plaice. Alternatively, the interchain disulfide bonds may be located *within* the basketlike inhibitory unit. This is possible if treatment with DTT results in the cleavage of disulfide bonds which are critical for the stabilization of the noncovalent interactions between the two adjacent inhibitory units. It is the disruption of these noncovalent bonds which would ultimately lead to the dissociation into two functional inhibitory units. Evidence to support this possibility is found in the disulfide-bond-stabilized M_r ~360 000 α-macroglobulin of lobster. It is therefore not possible to draw conclusions regarding the relationship of the interchain disulfide bonds to the basic inhibitory unit of α_2M given the available data.

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Kinetics and Mechanism of Transfer of Synthetic Model Apolipoproteins†

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ABSTRACT: The effect of hydrophobicity on the rate and mechanism of transfer of a synthetic amphiphilic peptide between phosphatidylcholine single bilayer vesicles has been evaluated. These peptides, which had the sequence C_n-SSLKEYWSSLKESFS (where C_n represents a saturated acyl chain of n carbons that is attached to the amino terminus of the peptide and n = 8, 12, or 16), were distinguished by the length of the saturated acyl chain of n carbons that was covalently bonded to the amino terminus. The transfer of the peptides was monitored by following the rate of change of the intrinsic tryptophan fluorescence that followed mixing of donor vesicles, which contained peptide, phosphatidylcholine, and a fluorescence quencher, with acceptors composed only of phosphatidylcholine. The transfer rates were independent of the structure and concentration of the acceptor. The kinetics were biexponential with the contribution of the fast and slow components being nearly equal. The rates of both components decreased with increasing acyl chain length; the respective free energies of activation were linear with respect to the acyl chain length. These results showed that, unlike lipid transfer, peptide transfer is not always a simple unimolecular process. However, like lipid transfer, the transfer rates are a predictable function of hydrophobicity. It is proposed that the peptides exist as dimers on the phospholipid surface and that the two components of transfer are due to sequential transfer of each molecule in a dimer.

In plasma lipoproteins, phospholipids, cholesterol, and proteins form a surface monolayer that separates the apolar core lipids from the surrounding aqueous phase (Shen, 1977). The surface components transfer among lipoprotein surfaces whereas the core lipids, mostly triglyceride and cholesteryl esters, require specific transfer proteins (Ihm, 1980; Zilversmit, 1975; Massey, 1985). The mechanisms of spontaneous phospholipid and cholesterol transfer are fairly well understood; transfer occurs via rate-limiting first-order desorption of the molecule into the surrounding aqueous phase followed by a rapid diffusion-controlled transfer to an acceptor lipoprotein (Lund-Katz, 1982; Massey, 1982). This process is regulated by the hydrophobicity of the transferring species with other structural features having only an incremental effect (Massey, 1982; Pownall, 1983). The mechanism of transfer of the apoprotein component of lipoproteins is important because some are ligands for receptor-mediated endocytosis (Gianturco & Bradley, 1987) and activators of lipolytic enzymes (Fielding, 1972; LaRosa, 1970; Zorich, 1985).

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