Low Resolution X-ray Structure of Human Methylamine-treated α₂-Macroglobulin*

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The structure of methylamine-treated human α₂-macroglobulin (α₂M-MA), a 720-kDa tetrameric inactivated proteinase inhibitor from plasma, has been determined to a resolution of 10 Å. Data were collected with synchrotron radiation at 120 K, and phases were calculated by multiple isomorphous replacement and solvent flattening. A novel feature of the structure of α₂M is present in its proteinase-binding cavity, dividing it into two compartments. The potential sites for proteinase entrapment in these compartments are sterically restricted. The positions of the thiol groups appearing from the functional important thiol esters upon their cleavage have been determined. They are found at the walls of the compartments at the center of the structure. The overall structure of α₂M-MA is much more sphere-like than previously inferred from electron microscopy studies. However, several aspects of the structure are well described by recent three-dimensional reconstructions. Possible models for the monomer, the disulfide bridged dimer, and native α₂M are discussed.

Human α₂-macroglobulin (α₂M)1 is the best studied member of the class of proteinase-binding α-macroglobulins (for reviews, see Refs. 1–3). One subunit of α₂M contains 1451 residues of which eight are glycosylated. This subunit has a mass of 180 kDa (4). Two subunits form disulfide bridged dimers (5). Two such dimers make noncovalent contacts to form the 720-kDa functional tetramer.

The native form of α₂M can form complexes with various proteinases. This complex formation is initiated by specific limited proteolysis of the bait region (6) found at residues 667–705 (7). The cleavage of the bait region initiates a series of conformational changes in the α₂M subunits resulting in entrapment of the attacking proteinase inside the tetramer. The final result of these changes is the transformed form of α₂M. The native and transformed forms of α₂M appear in electrophoresis as the slow and fast forms of α₂M (6, 8). The native form of α₂M contains internal β-Cys–γ-Glu thiol esters, formed from Cys-949 and Glu-952 in each subunit. During complex formation, these thiolesters become activated, and this activation results in covalent binding of the proteinase primarily through ϵ-Lys(proteinase)–γ-Glu(α₂M) cross-links (9–11). The bound proteinase is still active, but it is only accessible to small substrates and inhibitors. Two small proteinase molecules the size of chymotrypsin, but only one large proteinase like plasmin, can be bound to α₂M (12). A final result of the conformational change is that sites in the C-terminal domains (residues 1314–1451) (13–15) become exposed for interaction with the cellular receptor for α₂M-proteinase complexes. This receptor has been found to be identical to the low density lipoprotein receptor-related protein (16–18). Incubation of α₂M with methylamine also leads to thiol ester deavacy and covalent binding of methylamine (9, 19). The conformation of the resulting molecule, α₂M-MA, resembles that of the fast form α₂M-proteinase complex (20, 21). In α₂M-MA the bait regions are intact but poorly accessible. Therefore, α₂M-MA is inactive in proteinase complex formation (9, 19), but the receptor recognition sites are exposed in a manner similar to that of transformed α₂M (22, 23).

Transformed α₂M resembles the Cyrillic letter (later referred to as the H-view) when studied by electron microscopy (EM) (24). Its dimensions are 180–200 Å, 120–140 Å, and 80–90 Å as estimated from projections of different orientations and from three-dimensional reconstructions (25–33). Receptor recognition sites are located at the tip of each of the arms of the H (27, 34). When studied by EM, native α₂M has various shapes, which among others resemble a twisted cross (3, 26), a doughnut (25), and a padlock (26, 35). It has recently been shown that all of these shapes correspond to one single structure (36). In a three-dimensional reconstruction of native α₂M, the dimensions of the molecule were estimated to be 200 and 140 Å (35) with an internal cavity of cross-section 40 and 60 Å. In addition, molecules probably representing intermediates in the transition from native to transformed α₂M have been observed (3, 37, 38). The proteinase(s) in the α₂M-proteinase complex appear to partially fill a large elongated cavity (27, 28, 31, 33), which seems to be empty in α₂M-MA (28–30, 33). The flexible bait regions (39, 40) are relatively close to the thiol esters (41, 42). The latter are probably located in the center of the molecule at the inner surface of the cavity (29, 30, 43).

Thus, the two key functional sites of each α₂M subunit have an internal location in the α₂M tetramer as also indicated by the location of cross-links in α₂M-proteinase complexes (11, 12).

To provide a model of α₂M at higher resolution than currently obtained by EM, we have initiated x-ray crystallographic studies of several crystal forms. Tetragonal bipyramidal crystals of α₂M-MA and of several α₂M-proteinase complexes diffracting to a maximum of 9–11 Å resolution were reported earlier (44). Hexagonal crystals of α₂M-MA were recently found to be suitable for structural investigations (45), although the
limited diffraction power would not allow a detailed structural analysis. Crystals of α₂-M-MA from other species (46) and of the homologous complement component C3 (47) show similar resolution limits even with the use of high intensity synchrotron radiation and data collection at cryogenic temperature. None of the crystal forms found so far has shown diffraction to better than 8 Å resolution.

In this paper, we describe the three-dimensional crystal structure at 10 Å resolution of human α₂-M-MA. The electron density at this resolution does confirm several of the results already obtained by EM. Because of a higher resolution than normally obtained by EM, it reveals additional features of the molecule. The positions of the thiol esters are now firmly located at the inner surface of the central cavity. The tetramer has strict crystallographic 222 symmetry. The molecule is shown to be much more spherical than earlier suggested by the EM structures. The electron density reveals a large structure within the central cavity. This cavity body has not previously been seen in any of the EM studies. The low resolution structure presented here does allow some speculations about a possible model for the structural transition from the native to the transformed form of α₂-M.

MATERIALS AND METHODS

Native α₂-M at least 95% active in terms of titratable SH-groups after reaction with excess trypsin was prepared as described previously (9). Three different heavy atom derivatives of hexagonal crystals of α₂-M-MA were prepared for data collection. The complex between α₂-M-MA and the mercury cluster compound TAMM (48), which reacts with the free thiol groups induced by the methylamination, was prepared by the following procedure. 50 mg of α₂-M in 0.1 M NaHPO₄, pH = 8.0, 2M-MA = 3.04, was made 0.2 M in methylamine, and pH was adjusted to 8.0 with 0.5 M Na₂PO₄. After 4 h of incubation at 20 °C, the protein was desalted at 4 °C on a Sephadex G-25 column equilibrated in 20 mM Tris buffer, pH 7.7. The pool from this column was made 10 mM in glycyglycine by the addition of 100 mM glycyglycine. The concentration of α₂-M-MA was 3.0 μM after this treatment. 1 mM TAMM solubilized in 100 mM glycyglycine was added to 1.1 times the concentration of free thiol groups assuming 100% activity. The pH resulting from these additions was 7.9 at 20 °C and was not further adjusted. The reaction mixture was incubated for 1.5 h at 20 °C and then made 10 mM in iodoacetamide and immediately gel filtered as above and concentrated in Centricon 100 cells (Amicon Corp.). The amount of mercury in iodoacetamide and immediately gel filtered as above and concentrated in glycylglycine by the addition of 100 mM glycylglycine. The concentration of mercury from these additions was 7.9 at 20 °C and was not further adjusted. The reaction mixture was incubated for 1.5 h at 20 °C and then made 10 mM in iodoacetamide and immediately gel filtered as above and concentrated in Centricon 100 cells (Amicon Corp.). The amount of mercury in these additions was 7.9 at 20 °C and was not further adjusted. The reaction mixture was incubated for 1.5 h at 20 °C and then made 10 mM in iodoacetamide and immediately gel filtered as above and concentrated in Centricon 100 cells (Amicon Corp.).

EM structures. The electron density reveals a large structure when contoured at 1σ. The resulting electron density map showed the molecule distinctly from the solvent with little density left in the solvent region when contoured at 1σ. Reflections between 35 and 10 Å were included for the TAMM and Ta₆Br₁₄ derivatives, while none was found for the TAMM derivative. Phasing statistics for the final round of MIR phasing are shown in Table I. All sites for the three derivatives were inside or very close to the molecular boundary in the density map. The electron density map was contoured at 1σ. Reflections between 35 and 10 Å were included for the TAMM and Ta₆Br₁₄ derivatives, while reflections in the range 35–12 Å were included for the PIP derivative. For the final round of solvent flattening 2168 MIR phased reflections at 35–10 Å with FOM > 0.2 were selected. Sixteen cycles of flattening followed by 12 cycles of combined flattening and phase extension. A solvent content of 10% was assumed in the solvent flattening calculations. The resulting electron density map showed the molecule clearly distinct from the solvent with little density left in the solvent region when contoured at 1σ. Reflections between 35 and 10 Å were included for the TAMM and Ta₆Br₁₄ derivatives, while reflections in the range 35–12 Å were included for the PIP derivative. For the final round of solvent flattening 2168 MIR phased reflections at 35–10 Å with FOM > 0.2 were selected. Sixteen cycles of flattening followed by 12 cycles of combined flattening and phase extensions produced 3201 reflections with mean FOM = 0.816. The final R-factor was 22.3%. The final electron density map was contoured at 1σ and used in the program O for the presentation in this paper. In spacegroup P6₂₂₂, the hand of the structure is similar to that presented in the three-dimensional reconstructions published by Bosslet and co-workers (31). A more comprehensive description of data processing and phase calculations is given elsewhere (55).

The electron density map was skeletonized with BONES (56). At the low resolution of this work, the skeleton obviously does not represent any recognizable secondary structures. However, it does give a useful representation of the electron density. The resulting skeleton was inspected together with the electron density to erase minor nonconnected parts. From the final skeleton, it was possible to isolate one molecule and to select the individual parts that could give suitable skeleton objects representing tetramers, dimers, and monomers. These skeleton objects and the map_cover option in program O were used to extract the relevant electron densities from the complete unit cell. The isolated densities were used to produce graphical presentations of the complete electron density of one molecule in the crystal. In order to compare the crystallographic structure with two recent EM reconstructions, model A (31) and model B (33), these models were contoured such that the longest dimension in the H-view was approximately equal to that of the x-ray structure. The models were finally aligned manually with the move_obj option in program O.

RESULTS

The Unit Cell—The unit cell (Fig. 1) of space group P6₂₂₂ or P6₃22, with a = b = 324.3 Å, c = 216.4 Å, contains three 720-kDa tetramers each centered on special crystallographic positions with 222 symmetry, thereby confirming the 222 symmetry of the tetramer (31). The asymmetric unit contains one monomer. The crystal contains two very large solvent channels with diameters of 190 and 40 Å along the crystallographic c axis (Fig. 1a). A third channel along the crystallographic (a + b) axis has a diameter of 90 Å (Fig. 1b). As described below, the

| Table I | Phasing statistics as output from final run of MLPHARE |
|---------|--------------------------------------------------------|
| **Derivative** | **Cullis R-factor (centric)** | **Cullis R-factor (acracentric)** | **Number of sites** | **Resolution (Å)** |
| TAMM | 0.64 | 0.72 | 1 | 35–10 |
| Ta₆Br₁₄ | 0.81 | 0.91 | 3 | 35–10 |
| PIP | 0.75 | 0.78 | 8 | 35–12 |
The molecule shown in Fig. 2a is a tetramer. The structure is that of a large sphere with a diameter of approximately 125 Å. The tetramer consists of four identical units related by 222 symmetry. These units pack around a crystallographic 2-fold axis (not shown). Therefore, it is straightforward to isolate the tetramer in the electron density of the unit cell.

In the H-view (Fig. 2a) and in the End-view (Fig. 2f), the tetramer is seen to be organized in three major laminar bodies separated by low levels of density. The central one, termed the midlayer, is not further subdivided in the figures and thus contains four copies of the same density. That it is a separate entity can easily be seen in the H-view sections (Fig. 2, a and b) and in the End-view sections (Fig. 2, f and g). In the H-view, the dimensions of the midlayer are approximately 20 and 120 Å (Fig. 2a). The midlayer is for the most part separated from the exterior bodies by a space of approximately 10 Å in thickness. The dimensions of the midlayer in the X-view are approximately 120 and 130 Å (Fig. 2d). In the End-view (Fig. 2f), the midlayer is located in a large cleft between the two exterior bodies. In this view, it is easily seen that it contains two elongated lobes packing together in an anti-parallel mode. Its wheel-like shape (Fig. 2d) reveals that the midlayer surrounds a large internal cavity and that it includes a cavity body. From the indentations in the density, one unique quarter belonging to one monomer can be proposed (Fig. 2d).

The two exterior bodies as seen in the H-view (Fig. 2a) have a thickness of about 50 Å and are identical because of the molecular symmetry. Each body contains pairs of identical parts that can be separated into three unique ones by low levels of electron density. These are termed the front, the back, and the RBD (Fig. 2a). The tetramer contains four copies of each due to the crystallographic 222 symmetry. In the X-view (Fig. 2c), the two exterior bodies with their RBDs located at the ends are crossed by an angle of 70°. The dimensions of one exterior body (including the RBDs) in this view are approximately 180 and 110 Å. The front part of an exterior body has a rather compact structure (Fig. 2, a, c, e, and g). The back part is much more loosely organized and contains several large depressions with openings toward the surrounding solvent (Fig. 2, b, c, and g). Two back parts pack together in the central part of one exterior body (Fig. 2, c and e). Two front parts and two back parts together make up the core part of one exterior body. This has a flat disc-like shape (Figs. 2, a, c, and f). The distance between two RBDs located at the periphery at the same end of the molecule is 120 Å (Fig. 2f). The RBD has the appearance of an almost closed ring (Fig. 2, a and c). It is connected to the back part (Fig. 2c), although its major mass is leaning over the front part (Fig. 2, c and f).

The Central Cavity and the Cavity Body—Two very obvious features of the interior of the tetramer of α2M-MA are the large central cavity and the cavity body (Fig. 2, b, d, and g). The central cavity is basically defined by the disc-like core parts of the two exterior bodies (Fig. 2, a, c, and f) and the outer ring of the midlayer (Fig. 2d). Many clefts and holes appear in the density making up the walls of the cavity. These would allow easy access to the central cavity for objects with a diameter less than 10 Å, while objects with a diameter larger than 20 Å would seem to have difficult access. The areas most open for access are found between the back and the midlayer as seen in the H-view and End-view (Fig. 2, a and f).

The cavity body is part of the midlayer (Fig. 2d) and consists of four identical units related by 222 symmetry. These units cannot easily be separated in the density. The cavity body has the shape of an irregular cylinder of dimensions 28, 18, and 52 Å. This cylinder is, however, squeezed on one side at the middle of the cavity body (Fig. 2b) to the extent that a hole appears (Fig. 2d). The irregular cylinder can thus be seen as composed of two distorted tetrahedrons sharing one side. Four small protrusions on the cavity body (Figs. 2, b and d) make the only
connections to the rest of the midlayer.

The central cavity creates a continuous cylinder of solvent around the cavity body (Fig. 2, b, d, and g). The most narrow part of this cylinder is around the equatorial region of the cavity body (Fig. 2, b and d). Two symmetry related compartments are seen on each side of the cavity body (Fig. 2d). Close...
to the cavity body they have an elongated irregular ellipsoidal shape (Fig. 2b). In Fig. 2b, the long axis of the ellipsoid is from lower left to upper right with a length of about 100 Å. The other axis has a length of around 40 Å. Seen in the H-view, the two symmetry related ellipsoidal compartments together form an irregular X-shaped cavity having a depth of about 80 Å. The angle between the two ellipsoidal compartments is approximately 70°.

In the X-view (Fig. 2d) the two ellipsoidal compartments are seen largely along their longest axis. The distance between the edge of the cavity body and the walls in this view is typically 30 Å (Fig. 2d). The cavity body prevents objects with dimensions larger than 15–20 Å to move between compartments. Behind the cavity body in the X-view (Fig. 2e), the central cavity has the shape of a mirror image of a letter S. This inverted S consists of two halves of the ellipsoidal compartments and thus makes a connection between them.

The Thiol Esters—The positions of the cysteines appearing after cleavage of the thiol esters are mapped into the structure by the positions of the TAMM clusters used as heavy atom derivatives. Their positions are found on the surface of the back parts close to the cavity body (Figs. 2, b, e, and g). The coordinates and the geometry of the TAMM clusters are given in Table I. The pairs TAMM1-TAMM2 and TAMM3-TAMM4 each face one of the ellipsoidal compartments with the connecting line approximately along their short axis (Fig. 2b). The TAMM positions are accessible for large objects within the ellipsoidal compartments. The coordinates of the clusters found during phase determination represent the centers of mass of the TAMM compounds. Thus, the position of the sulfur atom of the cysteine is on a sphere with a radius of 4.5 Å. This radius is the sum of the S-Hg bond length of 2.42 Å in the structure of Protease α57 and the 2.04 Å bond length for the Hg–C in the structure of TAMM58. If the thiol group is in a sterically hindered environment as indicated by fluorescence spectroscopy (see “Discussion”), the thiol group is probably on the part of the sphere overlapping with the wall of the cavity.

**DISCUSSION**

The complex α2M-MA reacts slowly with proteinases and is from a physiological point of view inactive (9, 19). However, it is recognized by the αM receptor (22, 23), and the structures of α2M-MA and α2M-proteinase complexes appear to be very similar in EM studies, where models of α2M-MA have been used as the basis for localization of the trapped proteinase(s) in α2M-proteinase complexes (28, 59). The present investigation provides new and more detailed information on (i) the overall structure of the α2M tetramer, (ii) the location of the receptor-binding domains of αM, (iii) the location of the thiol group of Cys-949 appearing as a result of thiol ester cleavage, and (iv) the detailed shape of the large cavity within α2M, which is the site where proteinases become trapped. Compared with crystals of most other proteins, the crystals of α2M-MA contain an unusually high amount of solvent (87%). As seen in Fig. 1, the unit cell contains three large solvent channels ranging from 190 to 40 Å in diameter. Interactions in the crystal between the individual tetramers only take place at four small lobes of electron density probably representing the RBDs. The core, which comprises about 90% of the total mass of the protein, is weakly connected to the RBDs. Another source of high flexibility of the core is indicated by the few connections between various parts of the tetramer that could well give additional loss of order in the crystal lattice. The high solvent content and the small area involved in crystal packing provides an explanation for the limited resolution of the data obtained. Attempts at crystallizing many different members of the α2-macroglobulin family over the last 5 years have all resulted in crystals diffracting to about 10 Å resolution (44–47, 55). It is thus likely that all these crystals have a packing and a solvent content similar to the one described here.

This study demonstrates that it is possible to determine the structure of a very large macromolecule at 10 Å resolution with currently available methods and software. This finding can be important for other attempts at determining crystal structures of large macromolecules or macromolecular complexes with molecular masses in the million dalton range.

**Structure of α2M-MA at 10 Å Resolution**—Previous EM studies have discussed the shape of α2M-MA on the basis of its three major orientations on EM grids or in vitreous ice, the H-view, the L-view (here called the X-view), and the End-view (3, 31, 33). While α2M-MA in the H-view (Fig. 2a) is seen as an elongated particle of dimensions 160 and 125 Å, the X-view (Fig. 2c) reveals that the particle is almost a sphere with a diameter of 120–130 Å to which four small bodies of dimensions 50, 25, and 45 Å are attached. These bodies most likely represent the C-terminal 138-residue RBD of each subunit for the following reasons: (i) EM studies employing monoclonal antibodies have revealed that RBD is located at the tips of the H-like structure (27). (ii) The external position and the weak connection to the core through a single stretch of density are compatible with the release of RBD by proteolytic cleavage at a single bond (13–15). (iii) A length of 135 Å is observed for the long axis in the H-view in an EM study of the core alone (34). This is in agreement with the dimensions found here. (iv) The 138-residue RBD has been predicted to be a β-barrel (60).

Superposition onto the terminal lobe of one molecule of the P2 myelin protein (61), which has a β-barrel structure with 131 residues, makes it likely that it constitutes a major part of RBD (not shown).

If RBD is flexible relative to the core, as indicated by the single connection, this may be important when an α2M-proteinase complex binds to two adjacent receptors at the same time as proposed earlier (62). The flexibility might relieve steric strain in the complex between one molecule of α2M-proteinase and one to three molecules of the α2M-receptor complex (63).

The Central Cavity of α2M-MA—The central cavity in α2M-MA consists of two highly irregular ellipsoidal compartments crossed relative to each other (Figs. 2, b, d, and g). This overall organization of the cavity was previously inferred from a three-dimensional reconstruction based on EM data (31), although the dimensions were somewhat different from those found in the present crystallographic structure. The large cavity body in the center of the cavity is a novel feature. In the H-view (Fig. 2b), the cavity body is only 18 Å wide in the horizontal direction and is covered by the midlayer at the  

| Name           | x    | y    | z    |
|----------------|------|------|------|
| Center of the tetramer | 81.1 | 140.4| 144.2|
| TAMM1-TAMM2 | 78.5 | 162.4| 136.0|
| TAMM1-TAMM3 | 83.6 | 118.5| 136.0|
| TAMM1-TAMM4 | 101.3| 149.2| 152.4|
| TAMM2-TAMM3 | 60.8 | 131.7| 152.4|
| TAMM2-TAMM4 | 44.2 | 43.3 |
| TAMM3-TAMM4 | 39.1 | 59.6 |
| TAMM4-TAMM2 | 31.0 | 77.2 |
Comparison of the X-ray Structure and the EM Models of $\alpha_2$-M-MA—Two three-dimensional reconstructions, model A (31, 43) and model B (33, 35), based on EM data with a maximum resolution of approximately 30 Å have been published. For model A, the H-view, L-view, and the end view of Fig. 2 should be compared with Fig. 2, a, and c, of this paper. Notice that the end-view of model A is rotated by 90° relative to the view in Fig. 2f. For model B, the front and end views of Fig. 10 should be compared with Fig. 2, a and f, of this paper.

The overall dimensions of model A are 144, 193, and 130 Å (31). Thus the reconstructed molecule is larger than the tetramer in the crystallographic structure, especially at the long axis in the H-view. In the H-view, model A describes the front and back features quite well. In the equatorial region, the model is too slim and missing the delicate structures around the back-back interface. In the X-view, model A suffers from the fact that the dimension in the vertical direction is too long compared with the crystallographic structure. This masks the spherical shape of the core. The rotation of the two exterior bodies relative to each other is 20° in model A (31). This is very different from the value of 70° found in the crystallographic structure. One major reason for this is that the external position of RBD is not observed in model A. In the End-view, the midlayer is described fairly well. Two small protrusions indicate the positions of the RBDs. Model A describes the overall shape and dimensions of the central cavity very well in both the H-view and the X-view, although the cavity body is missing. Finally, the ellipsoidal compartments with axes of 135 and 35 Å in this model are more elongated than found here (31).

The overall dimensions of model B are 118, 150, and 103 Å (33). The model does not have internal 222 symmetry. In the H-view model B is rather smooth on the surface such that the front and back features are missing, and this model is also too slim in the equatorial region. This model too does not describe the external position of RBD. The relative rotation of the two exterior bodies is again significantly smaller in this model than in the crystallographic structure of $\alpha_2$-M-MA (33). In the End-view, the midlayer is present, but with an orientation different from that in the crystallographic structure. The internal cavity in model B is funnel-shaped with a narrow waist in the center (33, 35). The crossed ellipsoidal compartments observed in both model A and the crystallographic structure are not present in the model. The dimensions of a parallel reconstruction of the $\alpha_2$-M-chymotrypsin complex are 138, 175, and 125 Å (33, 35). Hence, the overall dimensions of the $\alpha_2$-M-chymotrypsin reconstruction appear to be closer to those found in the crystallographic structure of $\alpha_2$-M-MA.

The Thiol Esters—The thiol groups appearing after the cleavage of the thiol esters have been found by fluorescence spectroscopy to be in a sterically hindered but solvent accessible crevice at least 8 Å deep (41, 64). Their internal distances were estimated to be between 26.1 and 43.4 Å, and their positions were proposed to be in the central region of the molecule lining the cavity (42, 65). There is very good agreement between the distances of the thiol groups in these studies and the internal distances between the TAMM clusters (Table I). The internal distances found in EM studies using large ligands bound to Cys-949 are significantly larger than those found here. However, the diameter of the gold cluster used for labeling Cys-949 in these studies is 27 Å (29, 30, 43). Steric hindrance may have locally deformed the protein thereby causing the larger internal distances.

It appears from fluorescence spectroscopy that Glx-952 is positioned within 10–25 Å from the thiol group of Cys-949 in transformed $\alpha_2$M (64). The maximum separation of two such Glx positions has been estimated to be 50 Å (10), which is not much larger than the separation between the TAMM sites (Table I). This indicates that the Glx residue is located at the surface of the wall lining the same ellipsoidal compartment as the thiol group of the corresponding Cys-949.

Putative Locations of the Bait Regions—The bait regions cleaved by proteases are thought to be flexible but compact structures with a similar conformation in native $\alpha_2$M and $\alpha_2$M-MA (40, 41). The distance between a bait region and a nitrooxide spin-label attached to the SH-group of Cys-949 has been estimated to be 11–17 Å (41). In view of the structure and, in particular, the positions of the TAMM clusters presented here, the bait regions probably face the central cavity. It is tempting to speculate that the cavity body contains the four bait regions since such a location would agree with the observed distance between the thiol groups and the bait regions.

The $\alpha_2$M-MA has a well-defined central cavity. However, docking experiments show that this cavity is unable to accommodate proteases in the size of, for example, chymotrypsin (not shown). The cavity body is the major obstacle to this docking since it occupies much space inside the cavity. Hence, it is likely that the $\alpha_2$M-protease complex and $\alpha_2$M-MA have different structures, especially within the central cavity, although they probably have the same overall molecular shape as they both have crystallized in the tetragonal crystal system with the same space group and with similar cell parameters (44). During complex formation, the cavity body might change its central symmetrical location or perhaps collapse to accommodate the trapped protease(s). An asymmetric location of the cavity body could create extra space for a trapped protease, while leaving the rest of the tetramer almost unchanged.

The Monomer and Disulfide Bridged Dimer—Accepting the divisions of the structure into the parts proposed here, the monomer must contain one front, one back, one quarter of the midlayer, and one RBD part. One RBD and its corresponding back part must belong to the same monomer. Only one of the quarters of the midlayer has good connections to these two parts. The remaining difficulty in constructing the monomer is to determine which one of two front parts is in the same monomer as a given back part. Thus, two different models for a monomer seem possible. A rather compact monomer (Fig. 3a) with good contacts between the front and back parts and a well-defined internal cavity is one possibility. The alternative is a more elongated monomer (Fig. 3b) where the internal cavity is less shielded. Since monomeric inhibitors, e.g. rat $\alpha_1$-inhibitor 3, which are capable of trapping proteases exist (2, 66, 67), the monomer presented in Fig. 3a appears most attractive. In an EM study of chymotrypsin-treated rat $\alpha_1$-inhibitor 3, the molecule is described as squarish (68) and may thus correspond to the model presented in Fig. 3a.

Irrespective of the actual shape of the monomer there are two ways of creating a dimer from two monomers. First of all, consider the midlayer (Fig. 2d). A dimer can be created either by a vertical or a horizontal cut. None of these two possible interfaces has large areas of contacts. However, the cavity body...
is squeezed along the horizontal line (Fig. 2b), and the contact areas in the ring are smaller along the horizontal line (Fig. 2a) than along the vertical line (Fig. 2f). Secondly, consider the front and back parts of the exterior body (Fig. 2c). The interactions found here appear to involve considerably larger areas than those in the midlayer and are therefore likely to contain the major part of the noncovalent interactions between monomers. Here again there are two possible lines of cutting the tetramer into dimers. One of these, from the lower left to the upper right corner, seems to run through the smallest contact areas. At the same time, this line is closest to the probable horizontal cut in the midlayer. By cutting along these two weakest interfaces, a proposal for a disulfide bridged dimer made from two monomers as in Fig. 3a is presented in Fig. 3c.

Although appealing, a proposal for a dimer based on the argument that it should have a weaker interface to the other dimer than internally between the monomers is of course not necessarily correct. However, a location of the disulfide bridges probably in the midlayer of the End-view (Fig. 2f) has earlier been suggested by Delain et al. (3). If dimers of native $\alpha_2$M induced by mild acidic treatment react with proteinase, half-molecules resembling the greek letter $\eta$ or the upper half of an H are produced (69). Both of these observations support the model of the covalently linked dimer shown here. On the other hand, the model given here is very different from the dimer presented in Fig. 8 of Ref. 35. This dimer is created by the alternative cut through the tetramer. Results from bivalent cross-linking of proteinases do not clarify this discussion of possible dimers. It has been suggested that such cross-linking involves one Glx:952 from each of two disulfide bridged dimers (70). However, other experiments suggest that bivalent cross-linking can occur not only between disulfide bridged dimers but also within a single dimer (11). Interpretation of these results are further complicated by the uncertainty in the exact location of the proteinases in the $\alpha_2$M structure.

**A Model for Native $\alpha_2$M**—In EM studies of native $\alpha_2$M, two different views, the padlock and the lip-view, are frequently observed (26, 32). These two views have been shown to be related by a rotation of 45° around the long axis of the padlock (32, 36). A three-dimensional reconstruction of native $\alpha_2$M has been published recently (35) that also indicates how the padlock and the lip-view are related to the structure of transformed $\alpha_2$M. The lip-view most likely represents the structure of native $\alpha_2$M in the H-view orientation of $\alpha_2$M-MA. If the lip-view is rotated 90° around a vertical axis, a projection with two crossed arms similar to the X-view (Fig. 2c) defined here appears as seen in Fig. 6 of Ref. 35. In the H-view, a cleft
running from lower right to upper left in \( \alpha_{M-MA} \) (Fig. 2a) is present in a similar orientation. The major differences between the reconstruction of native \( \alpha_{M-MA} \) and the crystallographic structure of \( \alpha_{M-MA} \) presented here are (i) reduced density for RBD in the H-view, the X-view, and the End-view, (ii) increased density above the midlayer in both the H-view and the X-view, (iii) a large hole in the center of the molecule in the H-view, thus providing good access to the interior.

An attempt to simulate the structure of native \( \alpha_{M-MA} \) based on the crystallographic model of \( \alpha_{M-MA} \) is shown in Fig. 4. In that model, the midlayer has been separated into its dimeric parts, and both have been translated vertically in the H-view. The RBDs have been rotated toward the midlayer as rigid bodies around their connections to the core. The positions of the front and back parts are not changed. This simulation gives a density distribution similar to that in the reconstruction (35).

In the simulation, the two halves of the cavity body have been separated together with the rest of the midlayer. However, the location of the cavity body could be unchanged in the center of the cavity. During the conformational change from native \( \alpha_{M-MA} \) to transformed \( \alpha_{M-MA} \), the RBDs could move as rigid bodies to the locations observed in \( \alpha_{M-MA} \), thereby exposing those surface patches responsible for receptor recognition that are buried in native \( \alpha_{M-MA} \) (27). The midlayer and the RBDs seem to be the most flexible parts in the \( \alpha_{M-MA} \) structure. It is thus plausible that these parts are involved in the conformational change. Furthermore, the locations of Cys-949 (see above) make it likely that a signal to trigger the transformation can be transmitted to the midlayer after cleavage of the thiol esters. The changes in the front and the back parts are probably not very large, as indicated by the similar overall architecture of the reconstructed native \( \alpha_{M-MA} \) and the crystallographic structure of \( \alpha_{M-MA} \). If this simulation is correct, the mechanism for the conformational change from native to transformed \( \alpha_{M-MA} \) would be considerably simpler than those considered earlier (3, 26, 35, 37).

The bait regions in native \( \alpha_{M-MA} \) are rapidly cleaved by many proteases (1, 2). This implies that they are readily accessible for molecules with overall dimensions of 40–50 Å. If the bait regions are located within the internal cavity (see above), the large hole in the center of the molecule observed in both the simulation (Fig. 4) and the reconstruction (35) would allow access to the internal cavity and to the bait regions for an attacking protease. If the bait regions are located within the cavity body and protrude toward the walls of the cavity, the bait regions in native \( \alpha_{M-MA} \) might provide steric hindrance for access to the thiol esters, as previously suggested (71). This could shield the thiol esters from large nucleophiles. The increased reactivity of the thiol esters after bait region cleavage would thus result from the breakdown of such a shielding.

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