Similar Architectures of Native and Transformed Human α2-Macroglobulin Suggest the Transformation Mechanism*

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The refined three-dimensional structure of native human α2-macroglobulin (α2M) has been determined by cryoelectron microscopy and three-dimensional reconstruction. New features corresponding to “sigmoid arches,” “basal bodies,” and “apical connections” were observed in the molecule. Since similar elements are found in the architecture of transformed α2M, the 2 volumes were aligned in three dimensions. In their common orientations, they show many similarities except near the openings of the central chamber. In the native conformation, these apertures are fully opened, allowing the proteases to access the central chamber of the molecule, while in the transformed structure, they are partially closed. These structures suggest that α2M conformational change involves a strong lateral compression and a vertical stretching of the native particle seen in its four-petaled flower view to produce the H view of the transformed form. A model of structural transformation, in which all the parts of the α2M molecule seem involved in the entrapment of the proteinases is proposed.

Human α2-macroglobulin (α2M) is a plasmatic homotetrameric glycoprotein (720 kDa) assembled from two pairs of disulfide-bridged 180-kDa identical subunits. This molecule inhibits endopeptidases by a “trap mechanism” (1). Proteinases are caught and internalized into α2M after a specific proteolysis of a “bait region” followed by hydrolysis of a β-cysteinyl-γ-glutamyl thiol ester bond (review in Ref. 2). The thiol ester cleavage induces a covalent binding of the protease mainly to the N-terminus of the bait region. For the exposure of receptor-binding domains (RBDs) (3) that are involved in the entrapment of the proteinases is proposed.

Several models were proposed to describe the structural transformation of human α2M. A first model described the molecule as a “hollow cylinder” whose bases were supposed to be open and closed in the native and transformed states, respectively (18). Despite its didactic quality, this model was partially based on erroneous data. Indeed, the two types of EM views that the model intended to explain did not belong to native and transformed α2M, as suggested by Schramm and Schramm (19), but to the H and end-on views of the transformed molecule, as demonstrated by Tapon-Bretaudière et al. (20) and Stoops et al. (21). A second model derived from the observation of human α2M blocked at an intermediate stage of transformation by cis-dichlorodiammineplatinium II (22, 23) proposed that the molecule is subjected to an elongation leading to the exposition of the RBDs. Relying on immuno-electron microscopy (24), another model proposed that the flat lateral walls result from the unrolling of cylindrical dimeric half-molecules. These models are contradictory and additional information is required to understand the structural transformation of human α2M.

In this paper, we present refined three-dimensional reconstructions of native and transformed human α2M that share enough structural elements to align them in common orientations. The comparison of both architectures suggests a new model for the structural transformation.

EXPERIMENTAL PROCEDURES

Sample Preparation and Cryoelectron Microscopy—Native human α2M was prepared from fresh frozen plasma (Centre National de Transfusion Sanguine, Paris) by Zn2+-affinity chromatography (25) and Ultrogel AcA 22 (Sepharose, Villeneuve la Garenne, France) filtration as a final step of purification. The transformed conformation was obtained by incubation of native α2M for 3 h with 200 mM methylamine, in a 200 mM Hepes buffer, pH 7.5, 50 mM NaCl. The excess of reagent was removed by gel filtration on Ultrogel AcA 34 (Sepharose). The sample was diluted at a concentration of 120 μg/ml in 20 mM Hepes buffer, pH 7.2, 50 mM NaCl and applied on a 300-mesh copper grid covered with a thin holey carbon film. After removing excess solution, the grid was rapidly frozen by the blotting method (26), using liquid ethane as freezing agent. Cryoelectron microscopy was done with a Philips CM12 electron microscope equipped with a low dose kit, an EUV electron gun, a Gatan cryo-stage, and a Gatan cryotransfer device. Each specimen field was imaged twice, under low dose conditions (less than 10 electrons per Å2) at ~2.5-μm defocus, with the grid, respectively, tilted at 45° and 0°. For these experiments, an acceleration voltage of 100 kV was used with Kodak SO163 electron microscope films. The exact magnification of the form, the molecule shows a D2 point-group symmetry and produces two types of EM views termed “end-on” and “H” views (10). It is composed of two “lateral walls” bound through a pair of semicircular arches termed “paddle” (11) or “interwall structures” (12). The native conformation remained uncharacterized until recently (13, 14). Its architecture also possesses a D2 point-group symmetry and produces electron microscope (EM) views termed “lip” (15), “paddock” (16), “cross” or “four-petaled flower” (17), and “eye” (13).

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The abbreviations used are: α2M, α2-macroglobulin; RBD, receptor-binding domain; EM, electron microscope; DOP, direction of projection.
microscope was estimated to a value of X 42525 on a catalase crystal test grid (97).

Digitization and Interactive Windowing—Micrographs were digitized on an Optronics P1000 microdensitometer, using a 25-μm square scanning aperture corresponding to 0.584 × 0.584 nm. The windowing of the particle images was performed simultaneously on the tilted (45°) and uniltled (0°) digitized micrographs, using an interactive selection program (28). At this step, the relative orientations of each pair of micrographs in terms of tilt angle, direction of the tilt axis, and translation were calculated from the coordinates of the particle images. The selected images were submitted to contrast inversion and normalization process as described earlier (8).

Image Processing—The digitized images were processed according to the method of "random conical tilt series" (29), originally designed to make use of the preferred orientations of single particles on a support and could not be taken into account for image processing. Another method to determine the threshold leaving apparent all the features of the reconstructed particles is to compute the histogram of densities of the reconstruction volumes (data not shown). Two peaks of densities are always observed, corresponding to vitreous ice (lower densities) and protein (higher densities). Here, for native and transformed complexes give characteristic EM views (Figs. 1, A and I). The native molecule produces symmetric (Fig. 1B) and asymmetric (Fig. 1, C and D) lip views, two eye views (Fig. 1, E and F), and two types of padlock views (Fig. 1, G and H). As previously observed (13), the four-petaled flower, and Φ views were rare and could not be taken into account for image processing.

RESULTS

Electron Microscopy

In the vitreous ice layer, the native and transformed α2M complexes give characteristic EM views (Figs. 1, A and I). The native molecule produces symmetric (Fig. 1B) and asymmetric (Fig. 1, C and D) lip views, two eye views (Fig. 1, E and F), and two types of padlock views (Fig. 1, G and H). As previously observed (13), the four-petaled flower, and Φ views were rare and could not be taken into account for image processing. Similarly, transformed α2M produces symmetric (Fig. 1J) and asymmetric (Fig. 1K) end-on views, as well as two types of H views (Figs. 1, L and M). Here again, the orientation termed "lateral view" or "L view" was not observed in the ice layer. All the types of EM views shown in Fig. 1, B-H and J-M, are average images of homogeneous images classes, resulting from particle selection, two-dimensional alignment, and automatic clustering of uniltled-specimen images.

Three-dimensional Reconstructions

For three-dimensional reconstruction, we used the following strategy. For each homogeneous image class of untilted-specimen images, the corresponding images extracted from the tilted-specimen fields were subjected to three-dimensional reconstruction. Then, for the native and transformed α2M, seven and four primary volumes were calculated from the homogeneous classes of EM views shown in Fig. 1, B-H and J-M, respectively. At this stage, the primary three-dimensional reconstruction volumes corresponding to native and transformed α2M were independently aligned in a common orientation and two merged volumes were calculated. The merged three-dimensional reconstruction volumes were then subjected to three cycles of three-dimensional projection alignment (31), a procedure allowing the refinement of the three-dimensional recon-
Estimating of the Resolution Limits and Checking the Angular Distribution of the Images

The three-dimensional projection alignment method states that once a reference volume is created, one can submit to the process any additional tilted- or untilted-specimen image corresponding to a projection of this volume. Moreover, the topology spheres help to visualize the directions of projections (DOPs) assigned to these images. Relying on this type of representation, one can remove from the process redundant images (corresponding to the same DOP), or include additional images in the image set to fill the gaps in the angular coverage (DOPs devoid of a corresponding image). Thus, the purpose of the topological selection is to reduce the volume deformations occurring when some types of EM views are missing (e.g. missing cone artifact) or overabundant.

Fig. 2 illustrates the refinement procedure applied to the three-dimensional reconstruction of native $a_2$M. For this refinement, we disposed of a reference volume resulting from the merged three-dimensional reconstruction of primary volumes calculated from the tilted-specimen images corresponding to the averages of Fig. 1, B-H. Then, we subjected to a first cycle of angular and translational refinement a set of 4529 additional images (1140 untilted-specimen images and 3389 tilted-specimen images obtained at a tilt angle of 45°) (Fig. 2, A-C). The topology sphere is almost completely covered (Fig. 2, A-C) and the histogram (Fig. 2C) clearly shows that the average number of images per DOP is far above one, especially near the poles of the sphere. At this point, a topological selection reducing the image set from 4529 to 2457 images was carried out to keep a single image per DOP (Fig. 2, D-F). One observes that the topology sphere (viewed from the pole in Fig. 2D and from the equator in Fig. 2E) is almost completely covered except in the equator area corresponding to the $\Phi$ view. The histogram of Fig. 2F, giving the average number of images per DOP as a function of the second Eulerian angle ($\theta$), shows that the topological selection has left one image per DOP everywhere except for $\theta$ angles in the range of 80° to 110°.

After the selection step, the 2457 remaining images were subjected to three additional cycles of angular and translational refinement. At each cycle, a three-dimensional reconstruction volume was computed and used as the reference for the next cycle. The topology spheres and histograms corresponding to the first and third cycles of refinement are shown in Fig. 2, G-I and J-L, respectively. One observes that the Eulerian angles assigned to the images change from cycle to cycle and that a number of images migrate from the equator to...
the term oblique body and keep the word “arches” to designate an element of the semicircular sigmoid bridges now visible between the two pillars. The surface representations of Fig. 3 also shows that a single structure explains all the EM views observed in vitreous ice. For example, the molecule oriented in its lip view (Fig. 3A) gives the padlock view when rotated by 45° around an horizontal axis (Fig. 3C) and the four-petaled flower view upon a further 45° rotation (Fig. 3E). Furthermore, from this four-petaled flower view, a 90° rotation around a vertical axis yields the Φ view (Fig. 3G).

Three main differences are observed between this three-dimensional reconstruction volume and the volume previously obtained (13). First, two arches that were not resolved at the lower resolution limit of the previous three-dimensional reconstruction are now clearly visible at the front and the back of the lip view (Fig. 3A). Second, the dimensions of the particle that were 18 × 18 × 15 nm in the previous volume are now 21 × 21 × 14 nm, so that the Φ view looks narrower. Third, the oblique bodies that appeared as compact structures with uniform diameter are now composed of two main masses (asterisks in Fig. 3E) separated by a central constriction. Since similar masses, termed “basal bodies,” occurred in the transformed molecule, we keep the same name to designated these substructures.

**Transformed α₂M**—A good agreement exists between the current volume (Fig. 3, B, D, F, and H) and the one previously obtained (8). The only noticeable difference concerns the dimensions of the particle in its H view (Fig. 3H) that are 15.2 × 23.3 × 13.4 nm, while the previous estimation was 14.4 × 19.3 × 13.0 nm.

Due to the new features observed in the three-dimensional reconstruction volume of the native α₂M, we aligned the native and transformed structures in common orientations and found that the lip view of the native α₂M (Fig. 3A) corresponds to the symmetric end-on view of the transformed molecule (Fig. 3B). In this disposition, the arches locking the front and the back of the native molecule in the lip view (curved arrow in Fig. 3A) are homologous in the transformed molecule to the interwall structures (12) (curved arrow in Fig. 3B). Moreover, the small arms bridging the interwall structures to the apex of the transformed molecule, that we termed “apical connections” (arrowheads in Fig. 3B), also have their counterpart in the native form (arrowheads in Fig. 3A).

A 45° rotation of the three-dimensional reconstruction volumes around a horizontal axis produces a padlock view in native α₂M (Fig. 3C) and an asymmetric end-on view in the transformed molecule (Fig. 3D). In these orientations, two types of apertures are observed in the same locations at the front and back planes of both structures. We designate the larger channel (native, 78 × 39 Å; transformed, 48 × 29 Å) as “c1” and the smaller (native, 12 × 10 Å; transformed, 27 × 16 Å) as “c2” (arrows in Fig. 3, C and D).

A further 45° rotation around an horizontal axis produces the four-petaled flower view in native α₂M (Fig. 3E) and the H view in transformed α₂M (Fig. 3F). At the front of the particles one observes that two basal bodies are present in both structures (asterisks in Fig. 3, E and F). Although the two particles share common features in this orientation, as a result of the structural transformation, the H view is taller and narrower (29 × 15 nm) than the four-petaled flower view (21 × 21 nm). This lateral squeezing of the transformed molecule, also visible when the particles are subjected to a 90° rotation around a vertical axis (Fig. 3, G and H), is the keystone of the proposed mechanism of the transformation described below. This rotation places native α₂M in its Φ view (Fig. 3G), and the transformed molecule in its L view (Fig. 3H). Here again, there is a

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**Fig. 3.** Surface representations of the refined three-dimensional reconstructions of the native and transformed α₂M, at a threshold leaving apparent 180% of the molecular volume. A, C, E, and G, native human α₂M oriented in the lip (A), padlock (C), four-petaled flower (E), and Φ views (G), respectively. B, D, F, and H, transformed human α₂M oriented in the symmetric end-on (B), asymmetric end-on (D), Φ view (F), and L view (H). In A and B, the curved arrows are superposed to arches, and the arrowheads point to apical connections. In E and F, asterisks label the basal bodies. In G and H, the straight arrow signal the pillar and the asterisks the basal bodies.

**External Shapes of the Volumes**

**Native α₂M**—The actual three-dimensional reconstruction volume of native human α₂M shares many common features with the previously published structure (13). As visible in the four-petaled flower view (Fig. 3E) the overall molecule comprises two vertical “pillars” on its sides and two “oblique bodies” located at the fore and back planes. Although in a previous report we used the term “oblique arch” (13), we prefer to employ...
good agreement between the two structures. Actually, the rhomboid lateral wall of the transformed \( \alpha_2M \) is equivalent to the vertical pillar of the native structure (Fig. 3G, arrow) plus the upper-left and lower-right basal bodies of the two opposite oblique bodies (Fig. 3G, asterisks). Despite these minor differences, the three 2-fold axes of the two forms are perfectly aligned, as visible in Fig. 3, A and B, E and F, and G and H.

**Inner Features of the Volumes Explored by Slice Extraction**

The structural relatedness of native and transformed \( \alpha_2M \) can also be recognized in the inner features of the three-dimensional reconstruction volumes. For this purpose, Figs. 4 and 5 show a series of slices and truncated volumes of the transformed and native molecules cut perpendicularly to their three 2-fold axes with front, central, and back slices in rows 1–3, 4, and 5–7, respectively. A one-voxel thick slice corresponding to the cutting plane is displayed on the right side of each truncated volume. For example, Fig. 4, panel B, 4 contains two images describing the transformed \( \alpha_2M \) oriented in its H view. The left image contains a surface representation of the volume with the front half removed, while the right image corresponding to the cutting plane is the middle slice of the volume.

**The Interwall Structures**—These are described above in transformed \( \alpha_2M \) as two semicircular sigmoid bridges. In the end-on view, they form two S-shaped bridges at the front (Fig. 4, panel A, 2) and the back (Fig. 4, panel A, 6) of the molecule. In the H view (Fig. 4B), each interwall structure comprises five building blocks corresponding to an “arch,” two basal bodies, and two apical connections. Considering only the upper half-molecule, we find at the H view fore plane, that the interwall structure starts from the middle of the right lateral wall by a large mass termed “right basal body” (asterisk in Fig. 4, panel B, 1). Then, the arch starting from the basal body ascends toward the apex of the molecule between the two lateral walls (Fig. 4, panel B, 2). The arch, clearly appearing in the central slices as a high-density zone equidistant from the lateral walls (Figs. 4, panel B, 3–5), is linked to the right lateral wall apex by a thin apical connection (white arrowhead in Fig. 4, panel B, 3), then to the left lateral wall apex by a second thin apical connection (white arrowhead in Fig. 4, panel B, 4). Finally, the arch goes downward and reaches the left lateral wall (Fig. 4, panel B, 6) at the level of the left basal body (asterisk in Fig. 4, panel B, 7). These slices (Fig. 4, panel B, 2–6) help to understand why the interwall structures appear as two white dots between the lateral walls in the two-dimensional images of negatively stained transformed \( \alpha_2M \). The semicircular shape of the upper arch is clearly visible in the central slice extracted from the molecule oriented in its L view (curved arrow in Fig. 5, panel C, 4). In the same figure, one can see that the upper and lower arches join each other and form a central ring trapping the proteases in the central cavity.

The native \( \alpha_2M \) molecule possesses exact replicas of the interwall structures present in the transformed molecule. For
example, in the lip view they form two S-shaped bridges at the front (Fig. 5, panel A, 2 and 3) and at the back (Fig. 5, panel A, 5 and 6) of the molecule. In the four-petaled flower view, the upper interwall structure starts from the right basal body (asterisk in Fig. 5, panel B, 1), goes upward, reaches the apex of the molecule, then descends and merge the left basal body (asterisk in Fig. 5, panel B, 7). Near its apex the arch is connected to the pillars via right and left apical connections, as is the transformed molecule (white arrowheads in Fig. 5, panels B, 3 and 4). Pairs of high density masses are numbered 1, 3, and 4 in the lateral walls.

The Lateral Walls—The walls of the transformed molecule are particularly visible when the molecule is observed in its L view (Fig. 4C). In this orientation, four pairs of symmetrically disposed high-density areas form each lateral wall. In the front part of the L view (Fig. 4, panel C, 1), two high-density zones, termed masses No. 1 (Fig. 4, panel C, 1), correspond to two bumps on the front of the lateral wall (8). Underneath, three pairs of white spots, termed masses No. 2, 3, and 4, form a parallelogram (Fig. 4, panel C, 2). Masses No. 4 are located at the upper and lower vertices of the rhomboid, while masses No. 3 corresponding to the basal bodies project near the left and right vertices (Fig. 4, panel C, 3). Finally, masses No. 2 are located near the center of the lateral wall. A last feature specific of the transformed molecule is visible in the H view. In Fig. 4, panels B, 3 and 5, the masses No. 2 are linked by small bridges to the arches of the interwall structures (black arrowheads in the slices of Fig. 4, panels B, 3 and 5). We refer to these bridges as the “median connections.” They should not be confused with the apical connections that link the arches to the apices of the lateral walls (white arrowheads in the slices of Fig. 4, panels B, 3 and 4). To summarize, the apical and median connections link the arches to the masses No. 4 and 2 of the lateral walls, respectively.

In native α2M, although the lateral walls are incompletely formed, one can find some of the building blocks described above. For example, at the fore plane of the molecule oriented in its F view (Fig. 5, panel C, 1), the pillar is composed of two high-density zones that match well masses No. 1 (Fig. 5, panel C, 1). Underneath this first layer, the beginning of the upper and lower oblique bodies contains four high-density zones in a rhomboid disposition (Fig. 5, panels B, 3 and 4, respectively). The main difference between the interwall structures of the two forms is that in native α2M the upper and lower arches are more closely associated (Fig. 4, panel B, 1, and Fig. 5, panel B, 1).

FIG. 5. Internal features of the three-dimensional reconstruction volume of native α2M. The volume is placed in its three orthogonal orientations termed lip view (A), four-petaled flower view (B), and Φ view (C). The volume is seen from above and is gradually truncated from front (row No. 1) to back (row No. 7). For each truncated particle, the cutting plane corresponds to the white zone at the front of the surface representation, and the corresponding slice is shown on its right side. Asterisks represent basal bodies in B, 1 and 7, and white arrowheads signal apical connections in B, 3 and 4. Pairs of high density masses are numbered 1, 3, and 4 in the lateral walls.
Moreover, in the central slice the distance between the two lateral pillars is 108 Å (arrowheads in Fig. 5, panel B, 4), while in the transformed molecule the corresponding distance is only 47 Å (arrowheads in Fig. 4, panel B, 4).

The Central Cavity—This cavity of the transformed α2M molecule oriented in its H view comprises two superposed oblique chambers forming an X-shaped pattern. At the back of the molecule, a first chamber oriented lower-right/upper-left has an overall cylindrical shape with a diameter of 41 Å and a length of 184 Å (Fig. 4, panel B, 5). One median connection is present on each side of this cylindrical cavity (black arrowheads in Fig. 4, panel B, 5). At the front of the molecule, a similar chamber oriented lower-left/upper-right is also involved into a pair of median connections (black arrowheads in Fig. 4, panel B, 3). These chambers merge in the central slice and project as a “X” in the H view (Fig. 4, panel B, 4). The path of the hollow cylinders can also be traced along the molecule oriented in its on-end view. The two upper branches of the X-shaped cavity appear as two well separated dark zones (Fig. 4, panel A, 2) that become closer in slices located near the center of the particle (Fig. 4, panel A, 3), merge in the central slice (Fig. 4, panel A, 4), and separate again from each other in the lower layers (Fig. 4, panel A, 5 and 6).

In the native molecule (Fig. 5), the shape of the central cavity is more difficult to determine. The width of the central cavity is 108 Å (black arrowheads in Fig. 5, panel B, 4), while its maximum and minimum heights are estimated around 120 and 58 Å, respectively. The empty zone between opposed basal bodies is 64 Å in the central section of the volume oriented in its Φ view (arrowheads in Fig. 5, panel C, 4). If one looks for dark zones in the central slices extracted from the lip view (Fig. 5, panel A, 3–5), the cavity seems composed of two parallel short cylinders separated by a moderately dense central region. However, at the low threshold used for the surface representation no material is visible near the center of the molecule and the cavity appears as a single chamber (Fig. 5, panel A, 4). The structure of the central chamber can be understood when looking at the four-petaled flower view. Here again, a central low-density vertical column linking the upper and lower interwall structures is visible in the slices but not in the surface representations (Fig. 5, panel B, 4).

**DISCUSSION**

The observation of sigmoid bridges or interwall structures both in native and transformed α2M was decisive information for the alignment of these structures in common orientations. Figs. 4 and 5 show that the two particles share other features such as the basal bodies and the building blocks of the lateral walls (masses No. 1, 3, and 4). These data suggest a model for the structural transformation of human α2M based on the trap mechanism (1). That we termed the “accordion folding” phenomenon. To illustrate this hypothesis, we compare native and transformed α2M oriented in the four-petaled flower and H views (Fig. 6).

In the proposed mechanism, at first, one or two protease molecules enter the native molecule through the large c1 channel and interact with the bait regions. The thiol ester sites, located on the inner surface of the central chamber (12, 35–37) are activated and the proteinases become covalently bound through α2M(protease)-γ-Glu(α2M) bonds. Simultaneously, the structural transformation of the α2M leads to the physical entrapment of the still active proteases and to the exposition of the RBDs. For the sake of convenience, we limit our description to the structural transformation occurring around the upper-left c1 channel of the native form. In Fig. 6A, the c1 channel is surrounded on its left by the vertical pillar, on its right by the oblique body and the arch, and on its top by the left apical connection of the interwall structure. One sees that the front oblique body is composed of two basal bodies connected by a narrower central region (dashed line in Fig. 6A). The structural transformation reduces this contact zone to a small bridge at the center of a cleft in the corresponding region of the transformed α2M (asterisk in Fig. 6B). Simultaneously, the gap between the pillar and the lower-left basal body becoming filled (arrowheads No. 1 in Fig. 6, A and B) the shape of the lateral walls becomes rhomboidal. Finally, a new bridge (median connection) formed between the lateral wall and the arch partially closes the c1 channel (arrowheads No. 2 in Fig. 6, A and B).

The structural changes also affect the overall shape of the molecule. In Fig. 6, C and D, the front halves of the volumes are removed, and two broken lines joined by black dots follow the borders of the molecules (Fig. 6, C and D). In addition, the upper and lower ends of these broken lines are linked to the apices of the arches by dashed lines. This representation shows that the transformation of the molecule results from a lateral compression and a vertical stretching (Fig. 6C, horizontal and vertical arrows, respectively). In the native molecule, the upper segment of the broken line forms with the dashed line an angle of 110°, while in transformed α2M this angle is only 65°. This angular variation at the four edges of the H view resembles an accordion folding and explains the trap mechanism (1).
involve a major rearrangement of its subunits and/or a decrease of its global volume. This proposition is supported by the observation of electrophoretically "slow" and "fast" forms (38) and by the difference of sedimentation velocity (v20w) of 18.5 or 17.3 S to 20.5 or 19.2 S) observed for the native and methylamine-transformed α2M, respectively (39, 40). The frictional ratios fM of 1.55 or 1.67 found for the native structure and of 1.40 or 1.57 for the transformed molecule as well as the 10% decrease of the Stokes radius suggest that: (i) the transformed α2M undergoes a complete rearrangement of its subunits and/or (ii) it is more compact than the native form. Concerning the first point, the shape variations are difficult to express in terms of frictional ratios. In theory, at constant volume, the more a particle deviates from a sphere, the more its surface and its frictional ratio increase. This theory is verified for simple objects such as ellipsoids and rods. However, it is difficult to use for intricate architectures as those of the two forms of α2M with their cage-like structures and their central cavities opened to the outside by several channels. Concerning the second point, the difference of size between the reconstructed native and transformed molecules is in good agreement with the suspected compaction of the molecule. The overall dimensions of the native molecule are 210 × 210 × 140 Å instead of 152 × 233 × 134 Å for the transformed α2M. Thus, the transformed molecule is inscribed in a parallelepiped having lost 23% of its original volume. A more accurate measurement of this shrinkage was obtained by calculating the envelope of the reconstructed particles (data not shown). The outer shell of the native α2M circumscribes a volume of 13,500 voxels, while this volume falls down to 10,660 voxels for the transformed molecule, indicating a 20% contraction.

The x-ray structure of the methylamine-transformed human α2M has been recently determined to 10-Å resolution (9), and a model of native α2M was deduced from this structure. This attempt to simulate the structure of native α2M was based on the assumption that the lip view of the native form corresponds to the H view of the transformed molecule (14), an hypothesis in conflict with our reconstructions. A good agreement was found between the x-ray structure of the transformed molecule and our three-dimensional reconstruction volume. The two architectures possess rhomboidal lateral walls connected by sigmoidal bridges and a X-shaped central cavity. At the resolution of 10 Å, the crystallographic structure shows four small loops protruding from the lateral walls and presumed to correspond to the RBDs and a thin column of material termed "cavity body" within the central chamber. The limited resolution of electron microscopy may explain the fact that the cavity body and the RBDs were not resolved in this study. However, the cavity body is difficult to fit in proteinase-α2M complexes as its location in the central chamber corresponds to the space occupied by the trapped proteases (41, 42). Surprisingly, we observe a faint signal resembling the cavity body not in transformed α2M but in the native molecule (Fig. 5, panel B, 4). Such discrepancies raise the general problem of comparing structures obtained with techniques having different resolutions. Moreover, the structure of the particle can be suspected to vary with pH, ionic strength, and air-water interface forces in cryo-EM, with staining agent and sample dehydration in negative staining EM techniques, and with crystallization conditions in x-ray crystallography etc. The main features of the transformed α2M being observed both with cryo-EM and x-ray crystallography, small differences such as the presence or absence of a small linear structure like the cavity body may be considered as minor.

The presence of c1 and c2 channels in the native and transformed architectures give a new light on the possible interaction of the α2M with small ligands. For examples, there is numerous evidence for the interaction of α2M with various cytokines and growth factors. However, it is not yet clear whether these interactions result from a specific binding or are due to a nonspecific capture phenomenon. The intricate architecture of the molecule with c1 and c2 channels that resembles gel filtration beads could be propitious to the latter hypothesis.
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