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ELECTRON MICROSCOPE STUDIES OF HUMAN α_2–MACROGLOBULIN–CHYMOTRYPSIN COMPLEX: DEMONSTRATION THAT THE TWO STRUCTURES ASSIGNED TO NATIVE AND PROTEOLYZED α_2–MACROGLOBULIN REPRESENT TWO VIEWS OF THE PROTEOLYZED MOLECULE

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Electron microscope studies of native and protease–bound human α_2–macroglobulin have led to two contradictory models for these two structures. One viewpoint maintains that the native structure has the shape of)+(, which contracts on binding of the protease to the shape of (|). An opposing view proposes that the native structure has the shape of a padlock and that)+(and (|) are the side and end views of the proteolyzed molecule. In this investigation, electron microscope studies of the α–chymotrypsin–treated α_2–macroglobulin utilizing a tilt stage have shown that the two shapes [ )+(and (|)] interconvert. This demonstrates that these two shapes represent the side and end views of the proteolyzed α_2–macroglobulin which are related by a 90° rotation of the prototype molecule.

α_2–Macroglobulin isolated from human plasma is a glycoprotein (M_r, 718,000) composed of four identical subunits (M_r, 180,000) [1]. On exposure to an endoprotease, a limited proteolysis of α_2M occurs at a site called the "bait" region, resulting in a change in its structure [2–4]. This structural transformation results in the molecule becoming more compact as demonstrated by an increase in the sedimentation coefficient [5] and in the electrophoretic mobility [6] and by decreases in the radius of gyration [7] and the Stokes' radius [8].

The structures of native α_2M and α_2M–protease complexes have been examined by electron microscopy, and there are conflicting reports relating the various structures obtained by this technique to the native and protease–bound forms. Some studies [4,9,10] have suggested that the native form is described as a cyrillic character ( )+( ), which contracts to an ovate structure shaped like two crescents facing each other with a bar in the center (|) to give the "fast–migrating" form observed upon non–denaturing polyacrylamide gel electrophoresis. Speculative models of native and the proteolyzed α_2M have been proposed along with a mechanism by which the cyrillic form entraps the protease [11].

In a contradictory report, Tapon–Bretaudiere *et al.* [12] have suggested that these two forms [i.e., the)+(and (|)] represent the same structure viewed from the side and the end, respectively.

Abbreviation: α_2M, α_2–macroglobulin.

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This conclusion was derived from the observation that these two shapes appeared only after proteolysis of α_2M and were not detected in preparations of native α_2M. A more recent study demonstrated that native α_2M has the shape of a padlock, which image processing showed decreased from 19 nm to 16.7 nm on its major axis after binding α–chymotrypsin [13]. In this study, the α_2M–chymotrypsin complex contained both the)+(and (|) structures. The concordance between the sizes of the minor dimensions of the)+((11.8 nm) and the major dimension of the (|) (11.6 nm) further supports the proposition that the two shapes are side and end views of the same structure [13].

The excellent detail derived from electron microscopy of α_2M–protease complexes and the methylamine–reacted molecule have led to the development of several models representative of α_2M structure. The differences in these models result largely from the disagreement in relating various structures observed in the electron microscope to α_2M derivatives. The evidence used in supporting these opposing viewpoints is not convincing; consequently, we have carried out electron microscope studies of the protease–bound form of α_2M utilizing a tilt stage in order to determine the relatedness between the two shapes.

MATERIALS AND METHODS

Protein preparation. α_2M was prepared from fresh frozen plasma according to the method of Imber and Pizzo [14]. The amount of active α_2M was determined to be greater than 99% by measuring the number of sulfhydryl groups released during reaction with an excess of trypsin as described previously [15] and by analyzing the protein on SDS–PAGE and non–denaturing PAGE. The extent of the "fast–migrating" form of α_2M was routinely analyzed by immunoaffinity chromatography as described [16]. α–Chymotrypsin (Worthington, 3X crystallized, bovine pancreas) was prepared as a stock solution in 1 mM HCl, and the active–site concentration determined by titration with *p*–nitrophenyl acetate according to the procedure described by Bender *et al.* [17]. Protein concentrations were determined spectrophotometrically by using the following values for E^{280nm} and molecular weight, respectively: α_2M, 8.93 and 718,000 [18,19]. The active–site concentration of chymotrypsin was utilized to prepare the α_2M–chymotrypsin complex by reacting a 2.2–fold molar excess of chymotrypsin with α_2M for 15 min. Free protease was separated from the complex on a Bio–Gel A–0.5m column (1x40 cm) equilibrated in 50 mM sodium phosphate, pH 7.4. Active–site titration of the resultant complex revealed 1.6 moles of chymotrypsin per mole α_2M.

Electron microscopy. α–Chymotrypsin–treated α_2M specimens were deposited on Butvar 76–coated copper grids by the spray method as described previously using 0.25% methylamine tungstate as the negative stain [20]. The specimens were visualized with a JEOL 1200 electron microscope at an instrumental magnification of x75,000 with an accelerating voltage of 100 kV using conventional beam exposure. Under these conditions it was shown previously that the resolution is 30Å, which is adequate to distinguish the two shapes [13]. The possibility that either of these two shapes is an artifact resulting from beam damage was ruled out by the results of a dose series in which it was shown that the two shapes obtained at low dose (10 e/Å^2) is the same as that obtained by conventional beam exposure. The underfocus of the grid was 3000 – 5000Å. The images were recorded on Kodak SO–163 film.

RESULTS AND DISCUSSION

A number of studies have investigated the structure of α_2M and the proteolyzed derivative using electron microscopy. In earlier studies [4,9–11], the)+(structure was thought to represent native α_2M. However, in more recent studies, using well–characterized preparations of native α_2M, it has become apparent that these two structures do not occur until reaction of the molecule either with proteases or with methylamine. Since these two structures are only observed in preparations of α_2M following reaction with methylamine or with proteases, it has been assumed that they are related, possibly representing different views of the same molecule. A means of examining this proposition is to use a tilt stage to observe if the two forms interconvert.

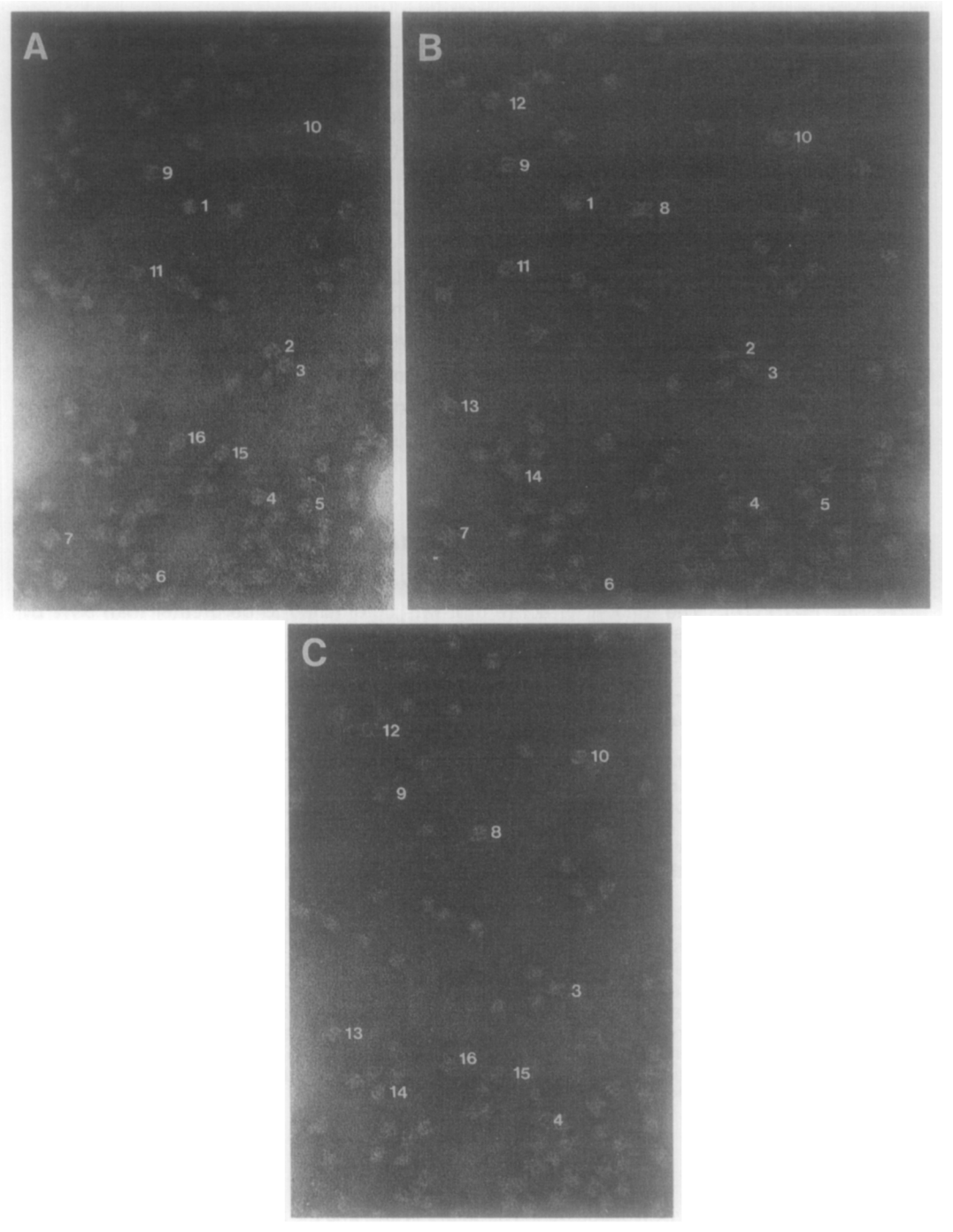

Figure 1. Micrographs showing the interconversion between the)+(and (|) of chymotrypsin–treated α_2M (x280,000). Panel A, B, and C are from 45°, 0°, and –45° tilted stages, respectively. The tilt axis is approximately perpendicular to the bottom of the panels. Molecules that interconvert are numbered (see text).

Figure 1 shows prints of the same field in which the stage was tilted 45°, 0°, and –45° in panels A, B, and C, respectively. The three views contain particles with the)+(and ovate–like shape (|). It is evident that as the stage is tilted, a number of these shapes interconvert. Those numbered 1–2,

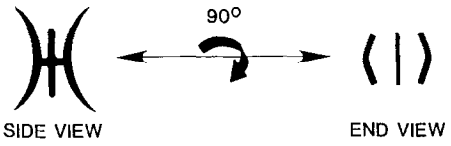

Figure 2. The arrow represents the tilt axis about which the two shapes interconvert. Molecules with a different disposition with respect to this tilt axis may undergo partial or no interconversion between these two forms.

5–7, and 11 interconvert between 45° and 0° perspective (panels A and B), while 8 and 12–14 interconvert between 0° and –45° (panels B and C). Those numbered 3–4, 9–10, and 15–16 interconvert between 45° and –45° (panels A and C). The views of the molecules in this last group are related by a 90° rotation of their structure. Most of the particles in the field do not interconvert between these two shapes. The result is expected since only those)+(and (|) shaped particles which have their major and minor axis, respectively, perpendicular to the tilt axis would be expected to interconvert (Figure 2). For those particles so arranged, there is a close correspondence between the minor dimensions of the side view and the major dimensions of the end view (10.7 nm, Figure 1). The interconversion of these two shapes has been documented for over 50 molecules. These micrograph prints show that a number of the)+(and (|) interconvert on tilting the particles only 45°. This probably results from the fact that the particles are in a variety of orientations on the grid film so that some are partially tilted in the direction in which the stage was tilted.

The interconversion between the)+(and (|) clearly demonstrates that the two shapes are the same structure related to each other by a 90° rotation of the prototype molecule. Therefore, the proposal that these two shapes represent the native and proteolyzed structures of α_2M is untenable. The demonstration that these two shapes represent the side and end of the same molecule gives considerable insight into the structure of the proteolyzed form of α_2M, and further indicates that the application of the samples to the grid film and the staining procedures used in this study have preserved the structure of the molecule. This would render these samples amenable to three–dimensional reconstruction studies using image processing [21]. The result of these studies should yield a reliable and more detailed model for the proteolyzed structure.

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