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Alpha-2-Macroglobulin: A Physiological Guardian

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Alpha macroglobulins are large glycoproteins which are present in the body fluids of both invertebrates and vertebrates. Alpha-2-macroglobulin (α_2M), a key member of alpha macroglobulin superfamily, is a high-molecular weight homotetrameric glycoprotein. α_2M has many diversified and complex functions, but it is primarily known by its ability to inhibit a broad spectrum of proteases without the direct blockage of the protease active site. α_2M is also known to be involved in the regulation, transport, and a host of other functions. For example, apart from inhibiting proteinases, it regulates binding of transferrin to its surface receptor, binds defensin and myelin basic protein, etc., binds several important cytokines, including basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), interleukin-1β (IL-1β), and interleukin-6 (IL-6), and modify their biological activity. α_2M also binds a number of hormones and regulates their activity. α_2M is said to protect the body against various infections, and hence, can be used as a biomarker for the diagnosis and prognosis of a number of diseases. However, this multipurpose antiproteinse is not "fail safe" and could be damaged by reactive species generated endogenously or exogenously, leading to various pathophysiological conditions.

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The body of animals has evolved in such a way that the threat to their well being is minimized. The primary mechanism of defense is innate immunity and alpha macroglobulins are an integral part of this system, and hence are evolutionarily conserved (Buresova et al., 2009).

Alpha macroglobulins are large glycoproteins which have been isolated from the hemolymph of invertebrates, plasma of vertebrates, and the egg white of birds and reptiles (Buresova et al., 2009; Raymond et al., 2009; Ma et al., 2010; Lim et al., 2011; Neves et al., 2012). The alpha macroglobulins family consists of α_1 macroglobulin (α_1M), α_2 macroglobulin (α_2M), complement components (C_3, C_4, and C_5), and pregnancy zone protein (PZP; Neves et al., 2012). Comparison of the amino acid sequence of C_3 and C_4 with α_2M indicates that they are evolutionarily related, as several patches of the protein sequence are very similar (Kaur and Katyal, 2011). The relationship could further be established by the presence of an internal β-cysteinyl-γ-glutamyl thiolester in C_3, C_4, and α_2M (Lin et al., 2008; Cole et al., 2009). However, activity of the thiol esters of these macromolecules differ toward small nucleophiles, as the thiol ester of C_3 incorporates glycerol while methylamine is incorporated by thiolesters of both C_3 and α_2M (Lin et al., 2008; Cole et al., 2009; Gai et al., 2009).

The alpha macroglobulin families of antiproteinases have a significant primary sequence homology with complement components C_3, C_4, and C_5 suggesting that these proteins arose from a common ancestor (Fig. 1). The hemolymph of *Limulus polyphemus*, one of the oldest surviving multicellular organisms dating back to more than 500 million years (Rudloe, 1979), contains a thiol ester containing protein which is part of the lytic system of hemolymph and proteinase inhibitory activity (Qin et al., 2010). This protein incorporates both glycerol and methylamine into its thiol ester and may be considered as a link between alpha macroglobulin proteinase inhibitors and the complement components C_3, C_4, and C_5. This protein exists as half molecule of present day α_2M and is a dimer of 185 kDa having disulphide-linked subunits.

Alpha-2-Macroglobulin: Structure

Alpha-2-macroglobulin is one of the major antiproteinase present in plasma of vertebrates. It is unique among other plasma proteinase inhibitors in its ability to inhibit virtually any proteinase, regardless of its specificity, and catalytic mechanism. It can inhibit proteinase present in the plasma as well as from other sources of origin (Khan and Khan, 2004; Lin et al., 2012). Vertebrate α_2M is a large tetrameric glycoprotein (M_r 725,000) consisting of identical subunits (M_r 179,000; Sheikh et al., 2003). Each subunit (human) contains 1,451 amino acid residues with glucosamine-based oligosaccharide groups attached to asparagines residues (32, 47, 224, 373, 387, 846, 960, and 1,401) and 11 intra-chain disulphide bridges (Kaur and Katyal, 2011). A reactive site is formed by thiol esterification of β-SH group of Cys-949 and γ-carbonyl group of Glx-952, which is susceptible to cleavage by denaturation or treatment with methylamine, resulting in covalent linkage of nucleophile to this cleavage site, forming γ-glutamyl methylamide (Fredslund et al., 2006; Lin et al., 2008; Cole et al., 2009; Aoun et al., 2011; Kaur and Katyal, 2011).

Alpha macroglobulins are composed of identical subunits, which could be monomeric (α_1-inhibitor 3 in rat and hamster, murinoglobulin in mice) or assembled in dimeric (human PZP, invertebrate α_2Ms) or tetrameric (vertebrate α_2Ms) structures (Table 1A). Table 1B shows some of the properties and characteristic features of human α_2M.

Located near the middle of the polypeptide chain is a unique sequence of amino acids which is susceptible to cleavage by almost all endopeptidases. Cleavage of this region, termed the "bait" region, triggers a conformational change in the structure of alpha macroglobulin and consequent entrapment of the

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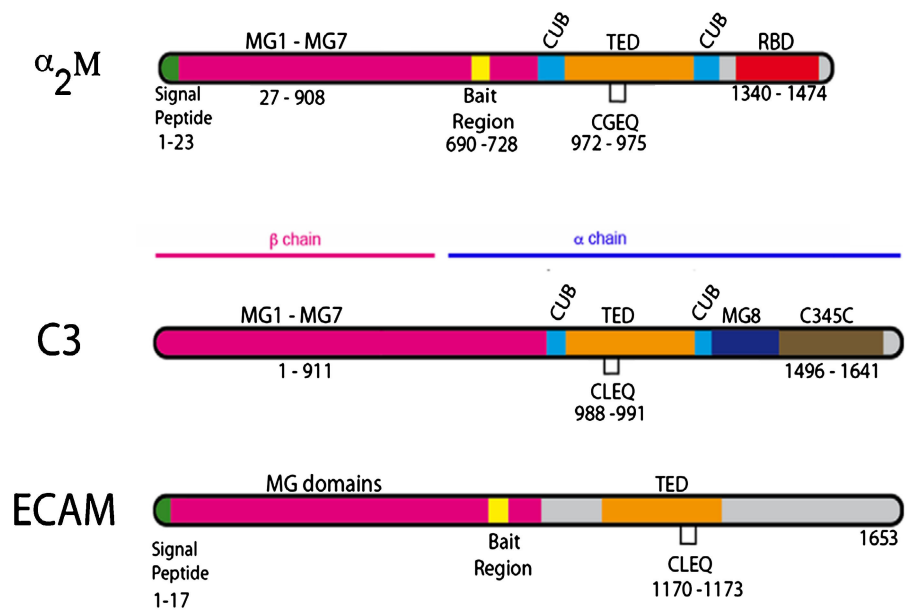

Fig. 1. A schematic representation of human α_2M, C3 and ECAM proteins. Domain assignments for α_2M and C3 are based on the respective crystal structures. The CLEQ sequence, a signature of the thioester bond, is present in all proteins. The C-terminus of ECAM displays low sequence similarity to that of C3 (*reproduced with permission*). α_2M, human α-macroglobulin; C3, C3 convertase; ECAM, *E coli* α-macroglobulin.

TABLE 1A. Some properties of α_2M from different organisms

Type	Size (Da)	Subunit size (Da)	Source	Thiol esters	Plasma level (mg/ml)	Refs.
Monomer	215,000		$Á_1$-inhibitor 3 in rat and hamster	+	4–10	Liu et al. (2007), Galliano et al. (2006)
	180,000		Murinoglobulin in mice	+	14.1	
Dimer	360,000	180,000	PZP	+	<0.01	Harrington et al. (2008), Brylev (2009)
	366,000	185,000	Limulus polyphemus (invertebrate) α_2M	+	0.5–2.0	
Tetramer	725,000	180,000	α_2M in vertebrate's circulation (human)	+	2–4	Sheikh et al. (2003), Lim et al. (2011)
	780,000	180,000	Ovostatin (in chicken egg white)	+/−	>1.0	

proteinase (Lin et al., 2012; Meyer et al., 2012). The cage formed by α_2M for entrapment of proteinases is a unique molecular structure (Marrero et al., 2012; Fig. 2). Following the conformational change, an internal β-cystenyl-γ-glutamyl thiol ester bond is exposed which has the potential to bind covalently to the entrapped proteinase or other nucleophiles present (Barrera et al., 2007; Lin et al., 2008). There are some receptor recognition sites on α_2M which are also exposed following cleavage of bait region and recognized by receptors present on several cell types. The inhibitor–proteinase complex is subsequently engulfed by these receptor-bearing cells and degraded, resulting in the clearing of proteinases from circulation.

TABLE 1B. The characteristic features of human α_2M

Properties	Human α_2M
Type	Homotetramer
Size (kDa)	720
Source	Blood plasma
Amino acids	1,451 (each subunit)
	5,804 (total)
Disulphide bonds	13
Secondary structure	
(a) Beta strands	67
(b) Alpha helix	17
References	Neves et al. (2012), Lin et al. (2012), Marrero et al. (2012), Travis and Salvesen (1983)

The α_2M tetramer is considered as "Dimer of Dimers" and is composed of a pair of identical subunits joined by disulphide bridges and two such dimers are joined by non-covalent interactions to form a tetrameric molecule (Wyatt et al., 2012). Each α_2M subunit contains an exposed sequence of amino acids, located in the middle, which is highly vulnerable to cleavage by proteinases—the bait region. The primary site of proteolytic cleavage is located in the sequence [Arg^{681}-Val-Gly-Phe-Tyr-Glu], present in the bait region. The proteolytic cleavage could occur at any residue in this hexapeptide sequence. The additional proteolytic cleavage sites are also present at two other regions situated ~15 and ~27 residues away from the hexapeptide region (Bahhady et al., 2008). The structural transformation following cleavage of the bait region results in an increase in electrophoretic mobility (Shi et al., 2011), sedimentation coefficient (Habtemichael et al., 2011), decrease in the radius of gyration (Osterberg and Pap, 1983), and Stokes' radius (Akashi et al., 2008). This compact form of α_2M is called the "fast" form and the transformation is termed as "slow-to-fast" transformation, owing to its behavior in polyacrylamide gel electrophoresis (PAGE; Habtemichael et al., 2011; Shi et al., 2011). The entrapped proteinase is not inactivated but only sterically hindered from the access to the macromolecular

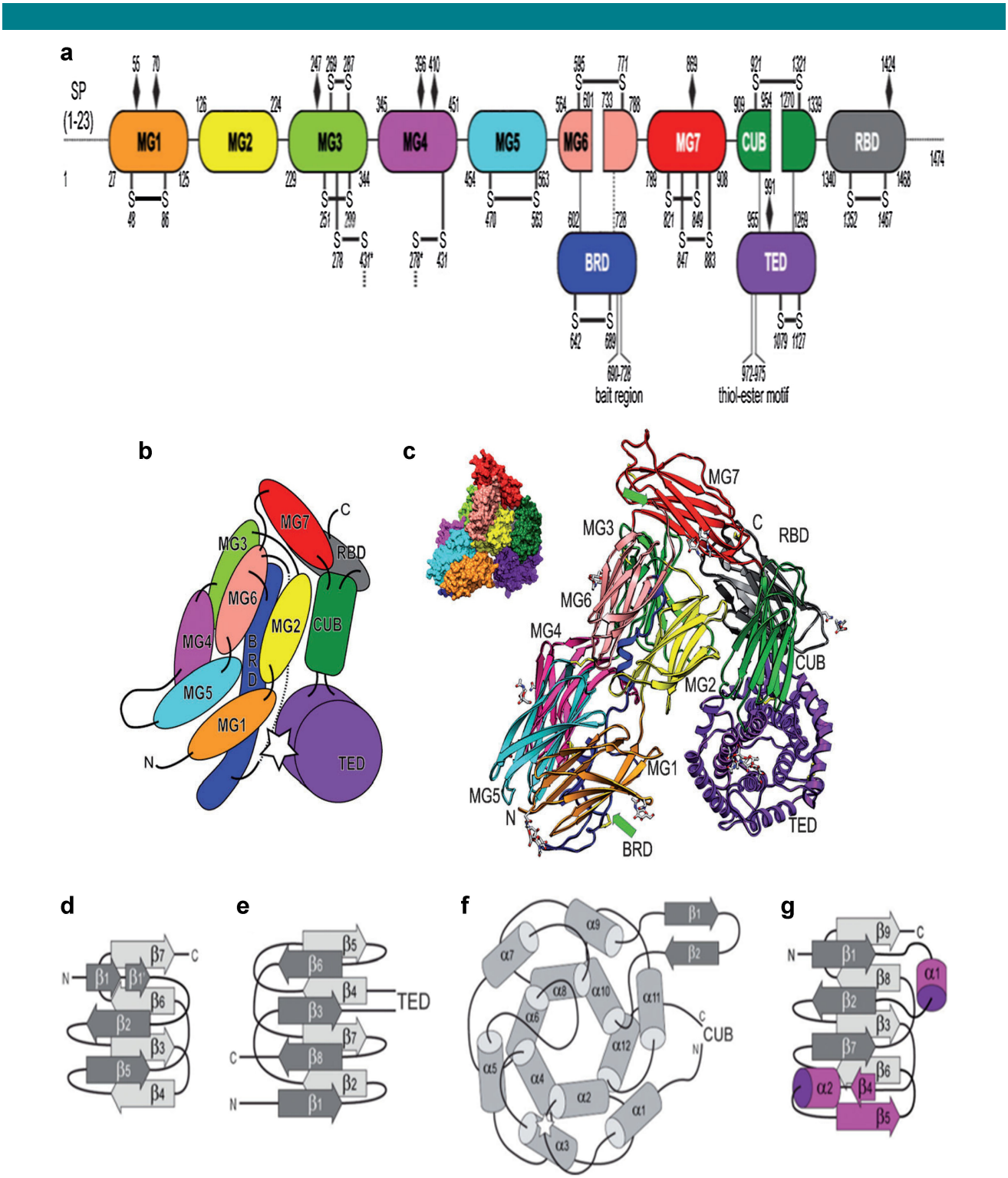

Fig. 2. Molecular structure of α_2M. a: Domain organization of human α_2M portraying the flanking residues of each domain, the disulfide bonds, the *N*-linked glycosylation sites, and the insertion sites of the bait region and the thioester. b: Approximate arrangement of the domains constituting the α_2M-methylamine (α_2M-MA) monomer in front view (left) and back view (right). c: Connolly surface and Richardson-type plot of α_2M-MA monomer in front and back view. Green arrows represent the anchor points of the flexible bait region. d–g: Topology scheme with secondary-structure element nomenclature of the MG domains (d), the CUB domain (e), the TED domain (f), and the RBD domain (g) (Marrero et al., 2012) (reproduced with permission).

substrates. The bait region is also believed to be necessary for non-covalent association of 360 kDa disulphide-linked dimers to give tetrameric α_2M. It also couples the cleavage of the bait region to the conformational changes that results in thiolester activation and finally trapping of proteinase (Gettins et al., 1995).

The five reactive sites of α_2M are briefly discussed here:

(a) *The Bait Region*: In the middle of each subunit is present an exposed 25 amino acid sequence which is highly susceptible to cleavage by endopeptidases of self or non-self origin. On cleavage of the bait region by a proteinase, an internal thiol ester is exposed, the cleavage of which result in conformational change in the structure of α_2M and entrapment of the proteinase. Trapping of proteinase does not block the active sites of enzyme, but only excludes it from access to large substrates.
(b) *The Internal Thiol Ester*: The thiol ester is formed between cysteine and glutamine, which is labile and readily cleaved by heat (Doan and Gettins, 2008), small nucleophiles, including primary amines, reductants, and water. Cleavage of internal thiol ester results in the formation of nascent α_2M with reactive glutamyl and cysteinyl residues in each of its subunit. Glutamyl residue may form a covalent linkage with lysine of proteinase and the cysteinyl residue may bind cytokines (Athippozhy et al., 2011) or A chain of the plant toxin, ricin (Pop et al., 2005).
(c) *The Receptor-Binding Site*: It is a 138 amino acid sequence present at the C-terminal of each subunit of α_2M. The receptor-binding site is exposed only after conformational change in the structure of α_2M, so that only the α_2M–proteinase complex is cleared, and not the native α_2M. The binding site is recognized by the 600 kDa α_2M receptor which is a cell surface glycoprotein (Strickland et al., 1990; Wyatt and Wilson, 2012), a member of low-density lipoprotein (LDL) superfamily (Pires et al., 2012; Wild et al., 2012).
(d) *The Transglutaminase Reactive Site*: A transglutaminase reactive site is present in close proximity to the bait region, 20 amino acids upstream from the primary proteinase cleavage site that is accessible in the native α_2M.
(e) *Metalloprotein*: α_2M is a metalloprotein and is a major zinc-binding plasma protein. Zinc is not required for the binding of proteinase, that is, antiproteinase activity, but is required for the binding of IL-1β.

The conformational change in the structure of α_2M upon reaction with proteinase results in the exposure of previously cryptic domain at the carboxyl terminal, called the receptor-binding domain (Holtet et al., 1994; Craig-Barnes et al., 2010; Petersen et al., 2010; Qin et al., 2010). The receptor-binding domain of α_2M is constituted by alternating β-strands and reverse turn, presumably forming a β-barrel type of domain. This domain is stable in the pH range 2.5–9.0 and is resistant to proteolytic digestion (Welinder et al., 1984). The domain is recognized by the cell surface receptors and the α_2M–proteinase complex is endocytosed, thereby removing the potentially damaging proteinases from circulation (van Leuven, 1984).

α_2M–proteinase complex is cleared from circulation primarily by receptors on hepatocytes (Travis and Salvesen, 1983; Bond et al., 2007; Armstrong, 2010), while in tissue it is removed by fibroblast cells, monocytes/macrophages, and syncytiotrophoblasts (Bowers et al., 2009; Ho et al., 2009; Ludvigsen et al., 2009; Craig-Barnes et al., 2010). The half-life of complexes is only a few minutes and they bind to receptors with high affinity ($K_d = 10^{-8} - 10^{-10}$ M). The affinity of receptors is similar for α_2Ms from all mammalian sources (Chiabrando et al., 2002; Mann, 2007; Bahhady et al., 2008; Bowers et al., 2009) and is not affected by the nature of the complexed proteinase. As reported by Van Dijk et al. (1992), activation of α_2M by methylamine induces a charge distribution on the protein which results in the uptake of the complex by the scavenger receptor on endothelial cells. It has been suggested that scavenger receptor might function as an additional system for the uptake of activated α_2M.

The mammalian receptor for proteinase-reacted α_2M is a low-density lipoprotein receptor related protein (LRP/α_2M-R; Strickland et al., 1990; Pandey et al., 2008; Fujiyoshi et al., 2011; Larios and Marzolo, 2012; Wyatt and Wilson, 2012) and belongs to the low-density lipoprotein receptor (LDLR) family (Borth et al., 1994; Truman et al., 2012). The LRP/α_2M-R is a 600 kDa glycoprotein that undergoes proteolysis in the trans-Golgi and is expressed as a non-covalently associated heterodimer of 515 and 85 kDa. It contain terminal complement type repeats, which are the ligand-binding domains, and epidermal growth factor precursor homology domain repeats that undergo an acid-inducible conformational change, allowing the ligand to dissociate from the receptor upon acidification of endosomes. In addition to α_2M, LRP/α_2 M-R has affinity for a number of other exogenous ligands with diverse biological functions implying that this receptor has various biological roles. LRP/α_2M-R is present on fibroblasts, hepatocytes, adipocytes, syncytiotrophoblasts, astrocytes, monocytes, and macrophages (Quigley et al., 1991). Apart from α_2M, it binds plasminogen activator-plasminogen activator inhibitor-1 (uPA/PAI-1) complexes, lipoprotein lipase, lactoferrin, *Pseudomona* exotoxin A, and apolipoprotein E-rich chylomicron remnant (Dupont et al., 2009; Dieckmann et al., 2010; Napolitano et al., 2012).

Mechanism of Action of Alpha-2-Macroglobulin

Despite having four subunits and each subunit having a bait region, the α_2M molecule has only two proteinase-binding sites, which are identical and independent (Pochon et al., 1981; Gerritsen et al., 2001). Each molecule of tetrameric α_2M can bind two molecules of smaller proteinases (such as α-chymotrypsin, trypsin; Lavalette et al., 2006; Shi et al., 2011) but only one molecule of larger proteinases (such as plasmin, a synthetic α-chymotrypsin dimer; Pochon et al., 1981; Barrera and Ramirez, 2006; Lavalette et al., 2006). The binding of proteinase to α_2M may be explained by the model given by Feldman et al. (1985) (Fig. 3), one of the first structural models of α_2M. Feldman et al. (1985) have presented a model of human α_2M that is compatible with most of the previous studies on the structure, function, and phylogeny of the protein. The model of the molecule resembles a hollow cylinder and crossing halfway between the ends of the molecules separated the inhibitor into two identical halves. Each half is made up of a ring with four arms (two per monomer projecting from one side). Each monomer constitutes one long and one short arm. The other side of the ring makes contact with second functional half. Trapping of the proteinases can occur by movement of one long arm per monomer and two arms per functional half. These long arms are termed as trap arms. As presented, the molecule has threefold axes of symmetry (one through the axis of the cylinder and perpendicular to it and each other in the median plane). The proteinase-binding sites are present on either side of the central rings aligned along with the central axis of the molecule. According to the model, the only conformational change required to affect a trap mechanism is a slight movement of the lateral arms of the molecule. Movement of the other two arms of the functional domain may occur, but is not required for trapping in the molecule. It is postulated that the thiol ester bond is located near the hinge of the trap arm of each subunit. Cleavage of the thiol ester bonds allows the trap arms to swing. In this model, reaction with amine can result in the same

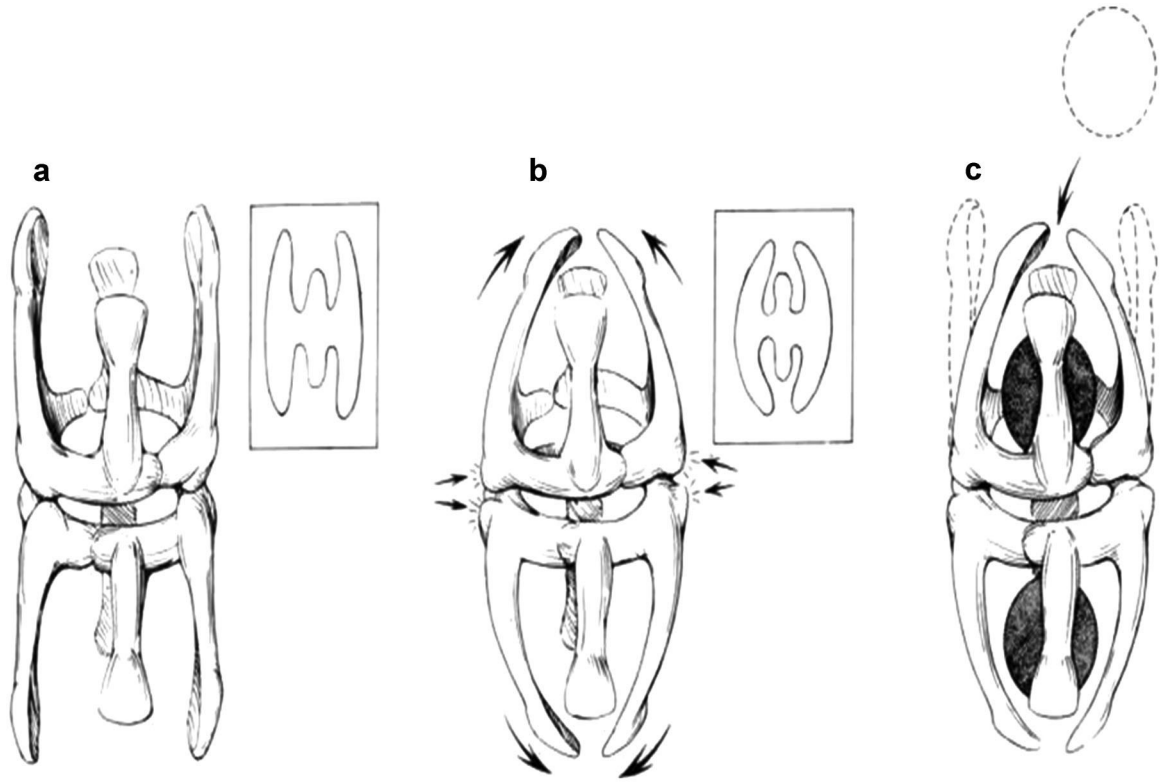

Fig. 3. Model of α_2M. a: Native α_2M, (b) the conformational change of α_2M (small arrows in the center indicate the positions for the receptor-recognition sites), (c) proteinase trapping by α_2M (Feldman et al., 1985) (reproduced with permission).

conformational change as that obtained by proteolytic cleavage of the bait region. The thiol ester within a pair are close together (at the base of adjacent trap arms separated by the plane between the functional halves), whereas the two pairs are located about 7 nm (diameter of the ring of the model) apart. The model further predicts that there is one recognition site present on each subunit (one per trap arm and two per half molecules) located at the interface between functional active halves. Trap closure results in the exposure of receptor recognition sites at the base of the ring near each swinging arm of the trap. This model also demonstrates why a large proteinase such as plasmin could not bind in 2:1 molar ratio (Gerritsen et al., 2001; Arandjelovic et al., 2007). Protrusion of the one of the domains of plasmin across the hollow core into the second binding site could stearically prevent the binding of a second proteinase molecule.

A fraction of bound proteinases form a covalent linkage with one of the thiol ester bonds of α_2M present on each subunit (Kaczowka et al., 2008; Lin et al., 2008). However, this covalent bond formation between α_2M and thiol ester bond is not critical for the stability of α_2M–proteinase complex (Kaczowka et al., 2008). Cleavage of the bait region exposes the internal thiol ester bond between Cys-949 and Glx-952 present in each identical subunit. Following cleavage of the bait region, thiol ester bond becomes activated and is susceptible to cleavage attack by nucleophiles. Cleavage of this linkage results in a change in conformation of α_2M and trapping of the proteinase (Gettins et al., 1993). A fraction of trapped proteinases also form a covalent linkage with the inhibitor through its ε-lysyl and γ-glutamyl of thiol ester (Lin et al., 2008). Change in consformation of α_2M results in an increase in its stability and reveals a receptor-binding domain (Qazi et al., 2000). The conformational change in structure of α_2M also leads to an exposure of carbohydrate residues. The α_2M–proteinase complexes are then rapidly cleared from circulation. As demonstrated by Imber and Pizzo (1981), the half-life of radiolabeled α_2M–trypsin and α_2M–methylamine complexes was 2–4 min when injected into mice, while that of radiolabeled native α_2M was several hours.

Alpha-2-Macroglobulin: Functions

The major function of α_2M is the inhibition (or specifically entrapment) of proteinases. It is unique in its function as it does not inactivate the proteinase, instead, it hinders the access of large molecular weight substrates to the active site of the proteinase. Amazingly, α_2M can trap almost any proteinase of all classes (serine, carboxyl, thiol, metallo-) (Roberts, 1986). The primary function of α_2M being the entrapment of proteinases, but its definitive function is, however, the delivery of proteinase to an endocytotic proteinase clearance pathway. Only fast form of α_2M (F-α_2M) is recognized and endocytosed by the cells of clearance pathway and the α_2M–proteinase complexes are cleared from the plasma rapidly (Idiris et al., 2003; Wang et al., 2011).

α_2M trap the proteinases released by granulocytes and other cells during inflammation and also regulate the extracellular proteolytic activity resulting from clotting and fibrinolysis. α_2M also protect the body against invasive pathogens as it may trap proteinases from non-human origins also. It is the only plasma inhibitor of specific proteinase of *Trichophyton mentagrophytes* (casual agent of ringworm), the neutral proteinase of *Fusiformis nodosus* causing ovine foot-root), the collagenase of *Clostridium histolyticum* (a gangrene organism), and proteinases of *Bacillus subtilis* (taken in by inhalation; Dolovich and Wichen, 1971).

Human chymase is a chymotrypsin-like protease stored in large amounts within the secretory granules of mast cells. Chymase secretion from stimulated mast cells influences tissue physiology in a number of ways. It may activate many important biological mediators like angiotensin, interleukin-1β, big endothelin, and interstitial collagenase. Chymase can also degrade basement membrane, inactivate the thrombin receptor, or stimulate the secretion of mucous from serous cells. It has been found that α_2M is an important inhibitor of chymase that regulate its activity (Raymond et al., 2009). Transferrin is the main iron transporting protein in the human blood which transports iron from absorption site (intestine) and storage site (liver) to the sites of utilization (mainly bone marrow). Cellular iron uptake is mediated by specific cell-surface transferrin receptors. It has been found that the binding of transferrin to the surface receptor is regulated by α_2M, along with α1-AT. It has been suggested that the pathophysiology of anemia during the acute-phase response of infections and malignant diseases could be explained by the inhibitory potential of α_2M and α1-AT (Roy, 2012). Prostate-specific antigen (PSA) is a serine protease chymotrypsin-like enzymatic activity and a member of glandular kallikrein family. It forms a complex with α_2M (Ezenwa et al., 2012). PSA reacts with α_2M more readily than any other protease inhibitor in the plasma (Kanoh et al., 2008).

α_2M also binds to a variety of other proteins. Defensins are highly abundant, variably cationic peptides that possess antimicrobial, cytotoxic, and chemoattractant properties and prepare mammalian phagocytes for participation in host defense and inflammatory processes. As reported by Panyutich and Gunz (1991), the fast form of α_2M is the principle binding protein of human defensin HNP-1 among the proteins present in plasma and serum. Native α_2M (slow form) bind only a little HNP-1. As fast form of α_2M is recognized and internalized by specific receptors present on macrophages and hepatocytes, hence, it is suggested that the α_2M may function as a scavenger of defensins and other peptide mediators in inflammed tissues and may constitute an important mechanism for the regulation and containment of inflammation. Following traumatic injuries or demyelination within the central nervous system, myelin basic protein is released into the circulation. This released myelin basic protein is highly immunogenic and it has been found that α_2M is the only major myelin basic protein-binding protein in human plasma. Myelin basic protein can bind to both fast and slow forms of α_2M. Binding to α_2M protect it from degradation by proteases such as trypsin and chymotrypsin. These observations suggest that in vivo α_2M might bind immunogenic myelin basic protein in extracellular compartments and help in its clearance from circulation (Gunnarsson and Jensen, 1998).

Tartarate-resistant acid phosphatase (TRACP) is an acid phophatase with an unknown biological function. TRACP circulate in human serum and is capable of generating reactive oxygen species (ROS) at neutral pH. There are two TRACP circulating in human serum, macrophage-derived TRACP 5a and osteoclast-derived TRACP 5b. TRACP 5b circulates in a large complex with α_2M. Two members of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family, ADAMTS-7 and ADAMTS-12, associates with and degrades cartilage oligomeric matrix protein (Liu et al., 2006a,b). As observed by Luan et al. (2008) α_2M is a novel substrate for ADAMTS-7 and ADAMTS-12, and more importantly, α_2M is the first endogenous inhibitor of ADAMTS-7 and ADAMTS-12.

Alpha-2-Macroglobulin and Cytokine Interaction

α_2M binds several cytokines, including basic fibroblast growth factor (bFGF; Peslova et al., 2009), platelet-derived growth factor (PDGF; Solchaga et al., 2012), nerve growth factor (NGF; Bramanti et al., 2007), IL-1β (Athippozhy et al., 2011), and interleukin-6 (IL-6; Nancey et al., 2008). The biological activity of some cytokines is inhibited when bound to α_2M, like that of IL-1β and bFGF, while some remain active (REF). The PDGF, NGF, and IL-6 remain partially active when bound to α_2M. Different cytokines bind with different specificities and to different degrees with native and transformed α_2M. The binding of NGF is the most efficient and least affected by cationic proteins. The binding of neurotrophin-3 (NT-3) is slightly affected by cationic proteins but is completely blocked by NGF. The binding of TGF-α, TGF-β_1, ciliary neurotrophic factor (CNTF), and IL-6 is severely blocked by cationic proteins/NGF (Barcelona et al., 2011).

A number of growth factors bind to α_2M at a site distinct from that where proteinases are entrapped, including members of transforming growth factor β (TGFβ) superfamily (Bowers et al., 2012). Some bind to native form of α_2M, for example, carboxypeptidase A (Valnickova et al., 1996), some to both forms, for example, TGFβ2 (Li et al., 2011) and some only to transformed α_2M, for example, growth hormone (GH; Fisker, 2006). The factors binding with native α_2M remain stable in the circulation, while complexes of factors with transformed α_2M are rapidly cleared from circulation via α_2M-receptors, hence, it is important to the physiology of the cytokine as to which form of α_2M they are getting bound.

Tumor necrosis factor-α (TNF-α) is a small polypeptide cytokine and an important multifunctional regulator of various cellular responses. It is produced mainly by monocytes and macrophages in response to various exogenous factors. Its biological activity may be influenced by α_2M as TNF-α bind strongly to fast form of α_2M (F-α_2M). TNF-α/α_2M–proteinase complex may be removed from circulation by the α_2M-receptor pathway (Jeng et al., 2011). The cytokine IL-1β binds to F-α_2M and retains its activity. Zn^{++} ions help in binding of IL-1β to α_2M (Athippozhy et al., 2011). IL-6 binds to α_2M and is carried by it in such a way that the activity of IL-6 is not lost and is also protected from the proteases, while free IL-6 is readily degraded. α_2M plays an important role in carrying IL-6 and making it available to lymphocytes, hepatocytes, and hematopoetic stem cells, resulting in the induction of the variety of host defense reactions, such as immune response, acute phase reaction, and hematopoesis.

Neurotrophins comprises of four neurotrophic factors—nerve growth factor β (NGFβ), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), which are structurally and functionally related proteins. Neurotrophic factors promote neuronal survival and differentiation during development, functional maintenance of neurons during normal homeostasis and play vital role in neuronal regeneration and response to injury. It has been found that neurotrophic factors bind to the α_2M at the sites primarily available on the F-α_2M (Wolf and Gonias, 1994). F-α_2M may be involved in regulating neurotrophin activity and/or its availability. Also α_2M may be the only major regulator of NGF-γ in plasma (Wolf et al., 1993).

The PDGF is a mitogen in serum for mesenchymal cells and a powerful chemo-attractant protein for inflammatory cells and cells involved in wound repair, thus important in normal inflammation and repair. Two molecules of PDGF bind covalently to one molecule of human α_2M. It is suggested that α_2M decreases the PDGF released locally at the site of inflammation (Solchaga et al., 2012). α_2M also form a complex with TGFβ forming the latent form of the complex (Hintze et al., 2012). The physiological role of TGFβ–α_2M complex may be the same as that of PDGF-α_2M interaction as both are simultaneously released from activated platelets at the site of inflammation. Inhibin and activin are members of the TGFβ superfamily with diverse functions in the body. Follistatin is structurally distinct from inhibin and activin, and bind these

proteins with high affinity. It has been found that activin and inhibin bind to α_2M (Schneyer et al., 1992; Vaughan and Vale, 1993; Jeppesen et al., 2012). Activin bind to both forms of α_2M (Niemuller et al., 1995), while, inhibin and follistatin bind significantly to only transformed α_2M (Philips et al., 1997).

In the cornea of eye, α_2M is localized in the epithelial, endothelial, and stromal cells and stromal extracellular matrix. α_2M protects the cornea from damage caused by proteinases synthesized by the cornea and those released from inflammatory cells and invading organisms (Ollivier et al., 2007). TGFβ is present in the eye and it may be involved in the etiology of major forms of cataract. TGFβ induces cataractous changes in rat lens epithelial explants. When the ocular media was assessed for the presence of inhibitors of TGFβ using a lens epithelium explants system in which TGFβ induces cataractous changes, α_2M was identified (Reneker et al., 2010). It blocks cataractous changes induced by TGFβ and protect lens cells from the damaging effects of TGFβ.

α_2M can bind to vascular endothelial growth factor (VEGF). The binding of α_2M to VEGF inhibits it from binding to its receptor. Binding of α_2M to VEGF is inhibited by heparin (Klettner and Roider, 2012). It is known that TGFβ$_1$ binds to α_2M. It has been found that transformed α_2M enhances TGFβ$_1$-induced growth responses and this effect is dependent on the binding of transformed α_2M to α_2M-receptor/LRP (Hneino et al., 2008).

α_2M binds a number of hormones. Hepcidin is a peptide hormone which plays a major role in iron metabolism. Hepcidin functions by regulating transmembrane iron transport. α_2M carries hepcidin in the plasma and is transferred to cells via receptor-mediated endocytosis (Peslova et al., 2009). Leptin, an obese gene product, is secreted from adipose tissue and controls the appetite and energy expenditure, thus regulating the body weight. It may inhibit synthesis and release of hypothalamic neuropeptide Y, which increases food uptake, decreases thermogenesis, and increases levels of insulin in plasma. In rodents and humans, the size of the body fat mass was found to be correlated with the level of leptin. It has recently been identified that the leptin-binding protein is α_2M, more precisely, F-α_2M. Binding of leptin to α_2M and its rapid clearance from the circulation significantly affect the bioavailability of leptin in human plasma (Birkenmeier et al., 1998).

Alpha-2-Macroglobulin: Pathophysiology

Concentration of α_2M in the serum also depends on the diet. Serum concentration of α_2M was compared in Thai vegetarians and omnivores. It was found that α_2M concentration in serum was higher in vegetarians than in omnivores. As suggested by Pongpaew and Boonyakarnkul (1994), higher α_2M values in vegetarians as compared with omnivores might be due to a mechanism which enables a body to adjust its requirement to intake by slowing down catabolic rates. In the process of cellular aging, the cellular intake of α_2M into the cells increases while it decreases during immortalization (Kondo et al., 2001; Table 2).

α_2M is known to inhibit a broad range of proteinases. It can also bind a number of growth factors, cytokines, ions, and lipid factors. Hochepied et al. (2002) showed that α_2M deficient mice are more resistant to a lethal Gram-negative infection, but are more susceptible to endotoxins. They suggested that α_2M plays a dual role during an acute phase response. It has a mediating role in establishment of a lethal Gram-negative infection by hampering the efficient clearance of bacteria while it also protect from endotoxic shock. Human group G streptococcus is part of the normal flora of the skin, pharynx, and female genital tract, but is also responsible for severe human infections. Strain G-148 expresses an IgG-binding protein called protein G. α_2M binds to the N-terminal region of protein G (Muller and Rantamaki, 1995). Protein G consists of repetitively arranged domains and binds α_2M irrespective of its conformation. In vivo and in vitro findings have demonstrated that the murine α_2M contributes to the resistance of mice to acute myocarditis induced by experimental *Trypanosoma cruzi* infection (Waghabi et al., 2002). α_2M also bind to GRAB (protein G-related α_2-macroglobulin-binding protein), an important virulence factor of group A streptococci (Godehardt et al., 2004).

F-α_2M shows an anti-apoptotic behavior regulated by expression of the low-density lipoprotein receptor protein (LRP; Fabrizi et al., 2001; Lee and Piedrahita, 2002). Intracellular parasites and host cells, both undergoes apoptosis on invasion of *T. cruzi* (Zhang et al., 1999; De Souza et al., 2006), and infected phagocytes undergo higher level of apoptosis as compared to non-phagocytic cells. De Souza et al. (2008) hypothesized that α_2M regulate the apoptosis triggered by the infection of peritoneal cells with *T. cruzi* in vitro. Macrophages known to display higher LRP levels displayed higher reduction in apoptotic levels during the F-α_2M treatment.

Receptors on macrophages recognize F-α_2M and the macrophages are rapidly stimulated to secrete eicosanoids. It has been suggested that the F-α_2M may be involved in the regulation of various functional responses of macrophages by stimulating them to release prostaglandin E_2 and other eicosanoids (Uhing et al., 1991). α_2M protect the tissues from acute injury related to inflammation not only by inhibiting the proteinases but in other ways also. As suggested by Nowak and Piasecka (1991), serum proteinase inhibitors, α_2M and α1 proteinase inhibitor, inhibit the generation of H_2O_2 from polymorphonuclear leukocytes. α_2M is also involved in clearing unfolded or misfolded proteins from extracellular spaces (French et al., 2008).

α_2M could be used as a marker for the diagnosis and prognosis of a number of diseases. The standard method of assessing liver fibrosis is liver biopsy which is invasive as well as risky. So, non-invasive serological biomarkers were studied and identified as α_2M, vitamin D-binding protein (VDBP), and apolipoprotein AI (Apo AI). α_2M is upregulated while VDBP and Apo AI are downregulated. The serum concentration of these biomarkers changes with different stages of fibrosis. Thus, these biomarkers could be used clinically to predict the stage of liver fibrosis without the need of liver biopsy (Ho et al., 2010). During liver fibrosis, TGFβ stimulate the production of matrix. It has been shown earlier that α_2M inhibit the activity of TGFβ. Tiggelman et al. (1997) observed that TGFβ-induced collagen formation by human liver myofibroblasts is reduced in vitro by activated α_2M. It has been hypothesized that in vivo α_2M may have an anti-fibrogenic effect on TGFβ induced matrix synthesis during liver fibrosis.

TABLE 2. Role of α_2M in various pathophysiological conditions

Condition/activity	Disease/pathology	Refs.
Downregulated	Rheumatoid arthritis in women	Brylev (2009)
Upregulated	Chronic liver disease, inflammatory joint diseases, multiple sclerosis, Binswanger's disease, nephrotic syndrome	Brylev (2009), Lionaki et al. (2011), Svenningsen et al. (2012)
Accumulation in cells	Aging	Kondo et al. (2001)

Cardiac isoform of α_2M is an early marker in cardiac hypertrophy and left ventricular mass in humans. Annapoorani et al. (2006) demonstrated that it could also be used as a marker for the diagnosis of myocardial infarcted diabetic patients and differentiating them from diabetic patients without myocardial infarction. Cardiac isoform of α_2M can also be used as an early diagnostic marker in HIV patients with cardiac manifestations (Subbiah et al., 2010). α_2M level is increased in the blood of patients with diabetes (Types I and II). Moreover, α_2M level in diabetic patients with diabetic complications was significantly higher than in the patients without complications (Turecky et al., 1999).

It has been found that patients who have undergone distal or total gastrectomy for gastric cancer have a significantly higher concentration of serum α_2M concentration in a depressed than in a non-depressed group (Fujita et al., 2003).

The concentration of complexed α_2M increases in pancreatitis. The more severe the attack, the higher the concentration of complexed α_2M has been found. This suggests that α_2M is not downregulated in the pathogenesis of acute pancreatitis (Meenakshisundaram et al., 2010). In patients with inflammatory bowel disease α_2M is excreted in high concentration (Becker et al., 1999). This key parameter could be used for diagnostic and prognostic purposes in these individuals. Measurement of α_2M along with myeloperoxidase and C-reactive protein in the urine provides a non-invasive and differential diagnosis of renal graft dysfunction (Nanda and Juthani-Mehta, 2009).

Human glandular kallikrein (hK2) is homologous to prostate-specific antigen and is a marker for prostate cancer. As demonstrated by Heeb and Espana (1998), α_2M, along with C1-inactivator, form complex with hK2 and inhibit it. α_2M–kallikrein complex, rather than free kallikrein, could play a role in the kinin release reported in some late-phase reactions, some instances of delayed type hypersensitivity and some cold-induced reactions (Lasser et al., 1991). α_2M and α_2M–proteinase complexes are also considered as functionally relevant biomolecules in male genital tract secretion (Obiezu et al., 2005).

α_2M can be used in vaccines as a delivery system and as an adjuvant. When proteinases attack α_2M, the thiol esters in each subunit become highly reactive to nucleophilic attack for a brief period of time. During this reactive state, α_2M thiol esters may incorporate may proteins including growth factors and hormones (Doan and Gettins, 2007). Utilizing this property of α_2M, it could be used as antigen delivery system (Bowers et al., 2012). α_2M act as an adjuvant and can increase the immunogenicity of the antigen several fold (Cianciolo et al., 2002). Techniques have been developed to incorporate non-proteinase antigens into α_2M (Kaczowka et al., 2008). Molecules other than peptides and proteins could be incorporated into α_2M and it can be used for drug delivery, especially to liver. It has been demonstrated in vivo, that α_2M is cleared in $<$10 min and 90% of this clearance is mediated by hepatocytes and Kupffer cells of the liver (Shibata et al., 2003; Bond et al., 2007).

Deficiency of antithrombin III (AT III) may lead to thrombosis. But it has been observed that thromboembolic events are rare in children as compared to adults with the same deficiency. Thrombin in AT III deficient children is inhibited by α_2M as its level is elevated throughout the childhood (Beheiri et al., 2007). While adults are unable to inhibit thrombin as they have low level of α_2M as compared to that of children. Moreover, even in the presence of AT III, α_2M is as important an inhibitor of thrombin as AT III (Spentzouris et al., 2012). α_2M (and von Willenbrand factor) possess *Asparagine*-linked ABO (H) blood group antigens in normal individuals with corresponding ABO phenotype. It has been suggested that during preparation of plasma materials for therapeutic use, ABO (H) blood group antigens in plasma glycoproteins should be considered (McGrath et al., 2010). Bond et al. (2007) incorporated guanosine triphosphate (GTP) into α_2M and developed a general approach to incorporate low-molecular weight substances into α_2M, which is of potential therapeutic application.

In conclusion, alpha-2-macroglobulin is a key member of alpha macroglobulin family and one of the major antiproteinases present in the plasma of vertebrates. It is unique among other plasma proteinase inhibitors in its ability to inhibit virtually all proteinase, regardless of its specificity and catalytic mechanism. It is unique in its function as it does not inactivate the proteinase, but hinders the access of substrates to the active site of the proteinase. α_2M can entrap proteinases from all the four major classes of endopeptidases (serine, carboxyl, thiol, metallo-). In mammals, α_2M predominantly exists as a homotetramer of 160–185 kDa subunits. Human α_2M ($M_r = 726{,}000$) is a tetramer of four identical ($M_r = 185{,}000$) subunits formed by the non-covalent association of two disulphide-bonded pairs of subunits. The four subunits are organized to form two protease binding sites in the tetrameric molecule that entrap and inhibit the proteases. α_2M is a large tetrameric glycoprotein, consisting of identical subunits. Located near the middle of the polypeptide chain is a unique sequence of amino acids which is susceptible to cleavage by almost all endopeptidases. Cleavage of this region, termed the bait region, triggers a conformational change in the structure of α_2M and the proteinase is entrapped.

Primary function of α_2M is the rapid inhibition of excess proteinases released during tissue injury as those liberated by neutrophils at the site of inflammation or exogenous sources such as those secreted by pathogens. It can hence, be a major contributor in protecting the body from wide disturbances which could result from uncontrolled proteolytic activity. The conformational change in the structure of α_2M upon reaction with proteinase results in the exposure of previously cryptic domain at the carboxyl terminal, called the receptor-binding domain. α_2M–proteinase complex is cleared from circulation primarily by receptors on hepatocytes while in tissue it is removed by fibroblast cells, monocytes/macrophages and syncytiotrophoblasts. The α_2M–proteinase complexes are cleared from the plasma quite rapidly.

α_2M has many diversified and complex functions, such as binding, transportation, and targeting due to the presence of multiple reactive sites. Each identical subunit of α_2M has five reactive sites: bait region, internal thiol ester, receptor-binding site, transglutaminase reactive site, and metal-binding site. Apart from being a major carrier for zinc, α_2M bind and regulate the activity of a number of proteins. For example, α_2M regulate the activity of chymase by inhibiting it, regulate the binding of transferrin to its surface receptor, form complex with PSA, binds defensin and is the only major myelin basic protein-binding protein in human plasma. α_2M acts as a binding and carrier protein for a large number of biologically important cytokines including TNF, PDGF, NGF, IL-6 among others. α_2M may also have a carrier and targeting role for these cytokines as they are delivered specifically to cells bearing α_2M receptor. The biological activity of some cytokines is inhibited when bound to α_2M, like that of IL-1β and bFGF, while some remain active. The PDGF, NGF, and IL-6 remain partially active when bound to α_2M. Different cytokines bind with different specificities and to different degrees with native α_2M and transformed α_2M. α_2M also binds to a number of hormones, such as, hepcidin, a peptide hormone involved in iron metabolism; leptin, an obese gene product; TRACP, capable of generating ROS in the serum; ADAMTS. α_2M is also involved in protecting the body from endotoxins produced by bacteria, regulating the apoptosis triggered by the infection with *T. cruzi*, inhibiting the generation of H_2O_2 from polymorphonuclear leukocytes, and clearing unfolded and

misfolded proteins from extracellular spaces. α_2M could be used as a marker for diagnosis and prognosis of many diseases, such as, liver fibrosis, cardiac hypertrophy, differentiating myocardial infarcted diabetic patients from diabetic patients without myocardial infarction, HIV with cardiac manifestations, etc. α_2M can be used in vaccines as a delivery system and as an adjuvant. Molecules other than peptides and proteins could also be incorporated into α_2M and it can be used for drug delivery, especially to liver.

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