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ORIGINAL PAPER

Acute phase proteins are major clients for the chaperone action of α_2-macroglobulin in human plasma

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Abstract Extracellular protein misfolding is implicated in many age-related diseases including Alzheimer's disease, macular degeneration and arthritis. In this study, putative endogenous clients for the chaperone activity of α_2-macroglobulin (α_2M) were identified after human plasma was subjected to physiologically relevant sheer stress at 37 °C for 10 days. Western blot analysis showed that four major acute phase proteins: ceruloplasmin, fibrinogen, α_1-acid glycoprotein and complement component 3, preferentially co-purified with α_2M after plasma was stressed. Furthermore, the formation of complexes between α_2M and these putative chaperone clients, detected by sandwich ELISA, was shown to be enhanced in response to stress. These results support the hypothesis that α_2M plays an important role in extracellular proteostasis by sequestering misfolded proteins and targeting them for disposal, particularly during acute phase reactions.

Keywords α_2-Macroglobulin · Chaperone · Protein misfolding · Acute phase proteins

Introduction

It is well established that a great deal of cellular energy and machinery is invested in protein homeostasis (proteostasis) systems. Compared to what is known about intracellular proteostasis mechanisms, however, our current understanding of the corresponding processes controlling proteostasis in the extracellular spaces of the body is very limited. This is striking given that protein misfolding and the extracellular deposition of misfolded protein aggregates are characteristics of many highly debilitating age-related diseases including arthritis, macular degeneration, type II diabetes and Alzheimer's disease (AD). Proteins are routinely exposed to stresses that are capable of inducing their misfolding including fluctuations in temperature and pH, oxidation and macromolecular crowding. Notably, extracellular proteins are exposed to an environment that is more oxidising than the cytosol (Ottaviano et al. 2008). Unlike their intracellular counterparts, extracellular proteins are also exposed to shear stress (i.e. the force exerted on soluble proteins and the extracellular domains of plasma membrane proteins as blood plasma is pumped throughout the body), which can induce protein misfolding (Bekard et al. 2011; Di Stasio and De Cristofaro 2010). Thus, the discovery and characterization of molecules that specifically recognise extracellular misfolded proteins, be they secreted chaperones (French et al. 2008; Humphreys et al. 1999; Yerbury et al. 2005), cell surface receptors (Jana et al. 2008; Husemann et al. 2002; Herczenik et al. 2007; Udan et al. 2008; Hespanhol and Mantovani 2002; Davis 1992) or elements of protease systems (Kranenburg et al. 2002), will shed important light on how proteostasis is maintained extracellularly and may help to uncover the causes of serious diseases.

α_2-Macroglobulin (α_2M) is a multifunctional protein that is best known for its role as a broad spectrum protease inhibitor (Sottrup-Jensen 1989; Borth 1992). Recently, it has been shown that α_2M has ATP-independent "holdase"-type chaperone activity (French et al. 2008), similar to two other known extracellular chaperones: clusterin and haptoglobin (Humphreys et al. 1999; Yerbury et al. 2005). α_2M is a large homotetrameric glycoprotein (720 kDa) that is

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formed by disulfide-linked dimers which non-covalently interact to give the quaternary structure (Sottrup-Jensen et al. 1984). At present, the structural elements responsible for the chaperone activity of α_2M are not known. High-sequence homology shared with complement component 3 (C3) has allowed for the prediction of the structure and location of homologous domains within α_2M, including a series of eight fibronectin type-3 macroglobulin domains (the last also being a receptor binding domain), an α-helical thioester-containing domain (TED) and a complement protein subcomponents C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein 1 domain (Doan and Gettins 2007). The mechanism by which protease inhibition is achieved by α_2M is well described and involves physical trapping of the protease as a result of large conformational changes that are initiated by protease cleavage of α_2M at the "bait region" (Barrett and Starkey 1973). Nucleophile containing side chains of the trapped protease react with the thiol ester of the TED domain of α_2M generating a covalent linkage (Sottrup-Jensen and Hansen 1982). Similar conformational changes in α_2M may be brought about by nucleophile attack of the thiol ester groups alone (Larsson et al. 1987). Transformation of α_2M by nucleophilic attack of the thiol ester results in the exposure of a cryptic binding site on each α_2M subunit for the low-density lipoprotein (LDL) superfamily receptor known as LDL receptor-related protein (LRP; also known as the α_2-macroglobulin receptor) and enhanced mobility when analysed by native gel electrophoresis (Imber and Pizzo 1981; Kaplan et al. 1981; Sottrup-Jensen et al. 1986; Kristensen et al. 1990). Via its interaction with LRP, α_2M facilitates the extracellular clearance of proteases (Feldman et al. 1983) and other non-covalently bound ligands, including cytokines (LaMarre et al. 1991).

α_2M is known to inhibit the formation of amyloid fibrils by a number of different proteins and peptides (Borth 1992; Narita et al. 1997; Motomiya et al. 2003; Adler and Kryukov 2007; Yerbury et al. 2009). It has also been shown that α_2M inhibits the stress-induced amorphous aggregation of client proteins in vitro (French et al. 2008). This latter activity involves the formation of stable, soluble complexes between α_2M and the misfolded client protein that can subsequently interact with a protease and then bind to cell surface LRP. These capabilities have led to the proposal that α_2M may play an important role in the clearance of extracellular misfolded proteins (French et al. 2008; Yerbury et al. 2009). α_2M is abundant in most extracellular fluids including blood plasma where it is present at between 1.5 and 2.5 mg/ml in adult humans (Ritchie et al. 2004). Similar to other members of the extracellular chaperone family, depletion of α_2M from human plasma renders plasma proteins more susceptible to aggregation and precipitation at physiological temperatures (French et al. 2008). We previously reported that at normal physiological levels of temperature and shear stress, human serum albumin (HSA), fibrinogen (FGN) and ceruloplasmin (CERU) are major client proteins for the chaperone action of clusterin in human plasma (Wyatt and Wilson 2010). The current study used similar methods to identify endogenous plasma client proteins for α_2M. Typically, investigations of chaperone activity are carried out using purified client proteins that can be induced to unfold at experimentally convenient rates. Investigation of extracellular chaperones within endogenous fluids, such as blood plasma, presents an opportunity to gain insight into their activity under conditions of direct relevance to their in vivo role. The identification in this study of disease-relevant proteins as major endogenous clients for α_2M supports the likely importance of its chaperone activity in protecting the body against serious diseases.

Results

It has previously been shown that incubation of citrated human plasma at 37 °C for 10 days with physiologically relevant shear stress (~36 dyn/cm^2) is sufficient to induce protein precipitation (Wyatt and Wilson 2010), and similar results were obtained for heparinized human plasma in the current study. Vascular shear stress reportedly ranges from 1 to 6 dyn/cm^2 in the venous system and 10–70 dyn/cm^2 in the arterial network (Malek et al. 1999); thus, in this study, we have used a value for shear stress that is approximately in the mid-range. As a result of ongoing protein misfolding and aggregation, plasma incubated with mild shear stress at 37 °C was significantly more turbid after 10 days than a sample of the same plasma held stationary at ambient room temperature ($p<0.001$; Fig. 1a). Size exclusion chromatography (SEC) analysis of protein isolated by Zn^{2+} chelate affinity chromatography (ZAC; as described in (Imber and Pizzo 1981)) from control plasma showed that around 80 % of the total protein eluted close to the expected mass of tetrameric α_2M (720 kDa); the remaining 20 % consisted mostly of lower molecular mass contaminants (<460 kDa) and a small amount of higher molecular mass proteins (>737 kDa; Fig. 1b). When proteins were isolated by ZAC from stressed plasma and analysed in the same way, similar to the control, most of the protein eluted close to the expected mass of tetrameric α_2M. The amount of lower molecular mass protein present was similar to the control; however, the proportion of higher molecular mass proteins detected was about three times greater. About 10 % of the total ZAC-purified protein from stressed plasma was comprised of species of molecular mass greater than that expected for tetrameric α_2M alone.

Reducing SDS-PAGE analysis of ZAC-purified proteins from control plasma showed that it mostly contained protein corresponding to the mass of reduced α_2M subunits (180 kDa; black arrow), together with a small amount of

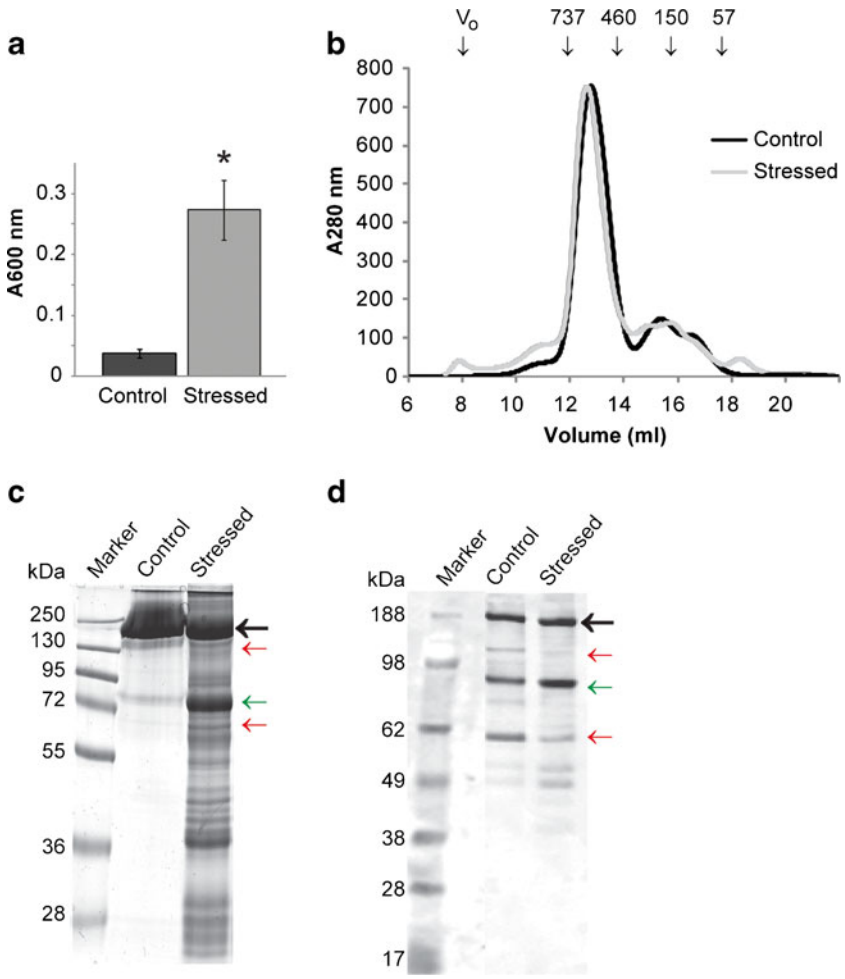

Fig. 1 Effects of shear stress on plasma protein aggregation and proteins co-purifying with α_2M. **a** Human plasma supplemented with 0.2 mg/ml sodium heparin, Complete™ protease inhibitor cocktail (EDTA-free) and 0.01 % (*w/v*) NaN_3 was subjected to shear stress, ~36 dyn/cm^2 at 37 °C for 10 days, or left at ambient room temperature. The precipitate from 200 µl was isolated by centrifugation and resuspended in phosphate-buffered saline, before the turbidity was measured spectrophotometrically using the absorbance at 600 nm (A600 nm). The values shown are mean±SD; n=3. *p<0.001 denotes increased A600 nm as determined by Student's t test. **b** Human plasma was processed by ZAC as previously described (Imber and Pizzo 1981) immediately following collection from the donor (*Control*) or after stress-treatment as described above (*Stressed*). Equal amounts of total protein were then subjected to SEC using a Superose™ 6 10/300 column. **c** After SEC to remove lower molecular weight contaminants (<460 kDa), approximately 40 µg of protein obtained by ZAC from the control or stressed plasma was separated under reducing conditions by 12 % SDS-PAGE. **d** Proteins isolated by ZAC from control or stressed plasma were separated under reducing conditions by 4–12 % SDS-PAGE (1 µg of total protein loaded per lane), transferred to nitrocellulose membrane and probed using anti-α_2M antibody. In **c**, **d**, the *bold black arrows* indicate bands corresponding to the intact α_2M subunit; the *green arrows* indicate bands corresponding to activated α_2M which has been proteolytically cleaved at the bait region; the *red arrows* indicate α_2M fragments generated by autolysis. In **c**, **d**, the sizes of pre-stained molecular weight markers (in kilodaltons) are indicated on the *left*. The results are representative of at least two separate experiments

protein having an apparent mass corresponding to that of the fragments formed when α_2M subunits are cleaved by proteases approximately in half at the bait region (85 kDa; green arrow) (Barrett and Starkey 1973; Fig. 1c). In contrast, similar analysis of proteins from stressed plasma indicated that the proteins obtained by ZAC contained the same two bands and a large number of additional unique bands (Fig. 1c). Western blot analysis confirmed that the 180 and the 85 kDa species purified by ZAC from control or stressed plasma were specifically detected by anti-α_2M antibodies (Fig. 1d). Bands at 120 and 60 kDa (red arrows) were also specifically detected by anti-α_2M antibodies and correspond to the expected size of fragments of α_2M that are known to be generated by heating (Harpel et al. 1979). Two additional bands close to 50 kDa were also detected by anti-α_2M antibodies and were more abundant in the ZAC-purified proteins from stressed plasma compared to those from control plasma. This suggests that some proteolytic cleavage of α_2M outside of the bait region occurred during the 10 day stress treatment despite the addition of protease inhibitors to the plasma at the start of the experiment. Notably, proteolytic cleavage of α_2M outside of the bait region only

accounted for a small number of additional bands; therefore, ZAC-purified proteins from stressed plasma contained many bands not attributable to α_2M.

Western blot analysis was carried out for 13 abundant plasma proteins to determine the relative amounts that co-purified with α_2M from control or stressed plasma. Of these proteins, CERU, FGN, α_1-acid glycoprotein (AGP) and C3 were more abundant in the total ZAC-purified protein fraction isolated from stressed plasma versus control plasma (Fig. 2a–d). For the remaining proteins, there was very little difference in their abundance regardless of whether plasma was stressed or not prior to ZAC; one example of proteins in this category, shown in Fig. 2e, is HSA. Western blots detected only monomeric (69 kDa) HSA in the ZAC-purified protein from control plasma but detected both monomeric HSA and a small amount of higher molecular mass species in the corresponding protein prepared from stressed plasma (possibly including covalently linked HSA dimers (Scorza and Minetti 1998)); however, the total amount of HSA was similar in both protein fractions (Fig. 2e). When the protein fractions obtained from stressed or control plasma were probed using anti-CERU antiserum, a single band was visible in the sample prepared from stressed plasma but was not detected in the protein fraction from control plasma (Fig. 2a). This band was smaller than that expected for intact CERU (200, 135 or 115 kDa; Sato et al. 1990) suggesting that this was a fragment of the protein. For FGN, bands corresponding to the approximate expected molecular mass of the intact α, β and γ chains were detected (69, 56 and 47 kDa, respectively) in the ZAC-purified fractions from both control and stressed plasma (Fig. 2b). The latter contained more of both the α- and γ chains; however,

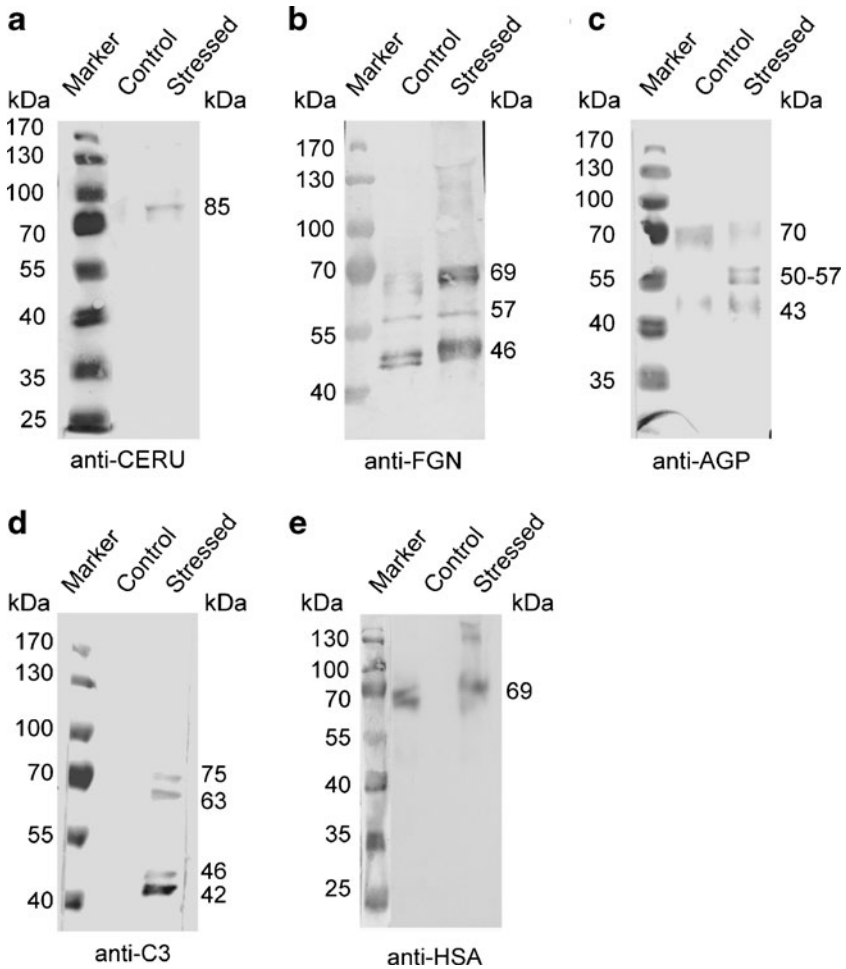

Fig. 2 Western blot analysis of proteins co-purified with α_2M by ZAC of control or stressed human plasma. Proteins isolated by ZAC were subjected to SDS-PAGE (40 µg of total protein loaded per lane), transferred to nitrocellulose membrane and probed using **a** anti-CERU; **b** anti-FGN; **c** anti-AGP; **d** anti-C3; or **e** anti-HRG antibody and the relevant secondary antibody. Western blots were developed using 3,3′-diaminobenzidine or enhanced chemiluminescence detection. Each panel shows the position of molecular mass markers and the specific protein species detected in each fraction. The *numbers to the left of the panels* are the molecular masses of the markers and the *numbers to the right of the panels* are the approximate masses of the bands detected in the protein fractions (both in kilodaltons). The results are representative of at least two separate experiments

similar amounts of the β chain were detected in both samples. The anti-FGN antiserum also detected a small amount of protein migrating at 100–150 kDa in the ZAC-purified protein fraction from stressed but not control plasma. While a band corresponding to the expected mass of AGP (43 kDa) was detected in the ZAC-purified fraction prepared from stressed plasma, and to a lesser extent in the corresponding sample prepared from control plasma, higher molecular mass species were also present (Fig. 2c). The highest molecular mass band around 70 kDa was actually more abundant in the protein fraction from control plasma; however, two bands closer to 50–57 kDa were clearly more abundant in the fraction purified by ZAC from stressed plasma. The band at 70 kDa and the two bands at 50–57 kDa may be highly glycosylated forms of the protein which can be found in low abundance in normal plasma (Poland et al. 2005; Theilgaard-Mönch et al. 2005). When the samples were probed with anti-C3 antiserum, four protein bands were detected exclusively in the fraction purified by ZAC from stressed plasma (Fig. 2d). The sizes of these bands did not correspond with either the intact α chain of C3 (115 kDa) or the α′ chain of C3b (105 kDa); however, smaller fragments detected at about 75 and 46 kDa could be the result of autolytic fragmentation of the α chain (Sim and Sim 1981). Conversion of C3b to the inhibited form iC3b is also likely to have contributed to the band at 75 kDa in addition to generating the bands at 63 and 42 kDa (Cunnion et al. 2004). To confirm that the four putative chaperone clients identified by Western blot analysis (CERU, FGN, AGP and C3) were bound to α_2M and not merely present as an artefact of ZAC, sandwich ELISA was used to detect α_2M–client protein complexes. On plates coated with anti-α_2M antibody, significantly higher levels of all four putative client proteins were specifically detected in fractions purified by ZAC from stressed plasma compared to unstressed control plasma ($p<0.05$; Fig. 3).

Discussion

The aim of this study was to identify endogenous client proteins for the chaperone action of α_2M in stressed human plasma, extending the work already carried out for the extracellular chaperone clusterin (Wyatt and Wilson 2010). Strikingly, all four putative clients identified in this study (CERU, FGN, AGP and C3) are major acute phase proteins, and as such their concentrations will be increased during conditions that may enhance protein misfolding (e.g. inflammation, increased local temperature, low pH, generation of reactive oxygen species). While α_2M expression is markedly increased during the acute phase in rats (Gehring et al. 1987), plasma concentrations of α_2M do not increase during the acute phase response in humans (Harpel 1976; Housley 1968). However, the plasma concentration of α_2M in healthy individuals is ~1,000-fold higher in humans compared to rats (1.5–2.5 mg/ml in humans (Ritchie et al. 2004) versus 12.7–18.4 μg/ml in rats (van Westrhenen et al. 2006)). Therefore, increasing the plasma concentration of

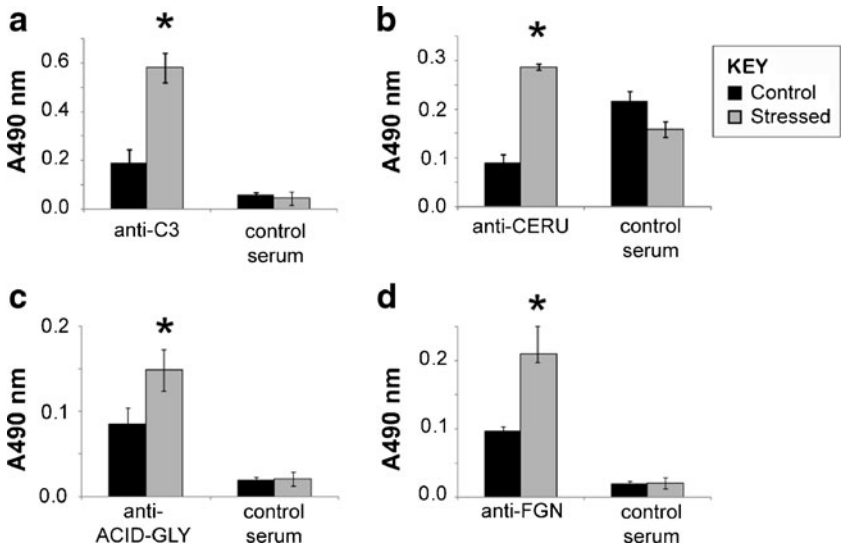

Fig. 3 Detection of α_2M–client protein complexes by sandwich ELISA. The wells of an ELISA plate were coated with affinity purified chicken anti-α_2M antibody and subsequently blocked using 1 % (*w/v*) BSA in PBS. Protein isolated by ZAC from control or stressed plasma was adjusted to 200 μg/ml total protein and added to the plate. The wells were subsequently probed using **a** anti-CERU; **b** anti-FGN; **c** anti-AGP; or **d** anti-C3 anti-sera or species-matched control sera, and the relevant secondary antibody (Online Resource 1). The results shown are the average absorbance at 490 nm (A490 nm; $n=3$, ±standard error) and are adjusted for non-specific binding generated in wells coated with control chicken IgY antibody. *$p<0.05$ denotes significantly increased A490 nm of wells containing stressed plasma relative to wells containing control (unstressed) plasma (Student's *t* test)

α_2M in humans may not be required in order to exert a similar protective effect to that possible during the acute phase in other mammals. Given that CERU and FGN have previously been shown to bind to clusterin in response to stress (Wyatt and Wilson 2010), the results of these studies support the hypothesis that extracellular chaperones are likely to play an important role in sequestering damaged proteins during the acute phase.

When proteins were purified from stressed and control plasma by ZAC (depleted by SEC of species less than about 460 kDa) and subsequently analysed by SDS-PAGE, a large number of bands not attributable to α_2M were uniquely detected in the sample prepared from stressed plasma; these unique bands represent proteins that only co-purify with α_2M from stressed plasma. The majority of protein isolated from stressed plasma by ZAC eluted close to the expected mass of tetrameric α_2M. This is consistent with previous reports that α_2M forms complexes with misfolded client proteins that are indistinguishable from α_2M alone by Superose^{TM} 6 SEC (French et al. 2008) and suggests that the capacity of α_2M to carry misfolded proteins is less than that of clusterin, which predominately forms very large complexes with amorphously aggregating misfolded proteins in which the mass of client protein "cargo" is approximately twice that of clusterin (Wyatt et al. 2009). However, SEC analyses showed that higher molecular mass species (including very large species that eluted at the void volume of the column $\geq 4\times10^4$ kDa) were approximately threefold more abundant in the proteins purified by ZAC from stressed plasma compared to those purified from control plasma. This suggested that larger complexes may have been formed between α_2M and client proteins in response to stress.

α_2M has been shown to inhibit both amorphous and fibrillar aggregation of a large number of client proteins (French et al. 2008; Yerbury et al. 2009). This study adds to our current understanding of the chaperone activity of α_2M by identifying the major proteins likely to interact with it in blood plasma as a consequence of physiologically relevant stress. It is expected that in healthy individuals, α_2M–client protein complexes are rapidly cleared from the bloodstream. If this mechanism was to fail or become overwhelmed (simulated by incubating plasma in the absence of cells), only then would α_2M–client protein complexes accumulate. In this study, only 13 of the many thousands of proteins present in human plasma were screened for their interaction with α_2M (see Online Resource); SDS-PAGE analysis supports that clients for α_2M may include numerous less abundant plasma proteins that were not identified in the current study (Fig. 1c). Given the limits in specificity of ZAC, as expected, contaminating Zn^{2+}-binding proteins were present in fractions purified from both stressed and control plasma; however, as shown for HSA (the main zinc-binding protein in plasma), there was little difference in their abundance in fractions purified from control and stressed plasma. In contrast, CERU and FGN, which are also known to bind zinc (Scott and Bradwell 1983; Marx 1988), were clearly more abundant in samples prepared from stressed plasma and could also be detected bound to α_2M in these samples by ELISA. It is perhaps unsurprisingly that CERU and FGN which have previously been identified as endogenous chaperone clients for clusterin (Wyatt and Wilson 2010) were also identified as endogenous chaperone clients for α_2M. It was recently shown that when αB-crystallin (an intracellular chaperone with holdase-type activity similar to α_2M and clusterin) was added to plasma from patients with multiple sclerosis, rheumatoid arthritis or amyloidosis, it bound to a common set of plasma proteins, and furthermore that this binding was enhanced when the plasma was heated to 42 °C (Rothbard et al. 2012). The set of αB-client proteins identified in Rothbard et al. (2012) included all four of the α_2M clients identified in the current study and suggests that these proteins are likely to have a higher propensity to misfold in response to stress compared to other plasma proteins.

CERU, the major copper-carrier protein in biological fluids, is known to be susceptible to denaturation and fragmentation as a result of mild stresses (Sedlák et al. 2008; Islam et al. 1995). This protein has been of interest in (AD) research owing to the fact that levels of non-CERU-bound or "free" copper correlate with cognitive decline in AD (Squitti et al. 2006, 2009). Furthermore, the levels of apoceruloplasmin (lacking copper) are higher in AD patients compared to normal controls (Brewer et al. 2010), directly implicating CERU in AD pathology. Since levels of free serum copper are positively correlated with the extent of fragmentation of CERU in AD patients (Squitti et al. 2008), the apparent specific binding of α_2M to fragmented CERU in this study is particularly interesting and warrants further investigation. Dissociation of copper from CERU has been implicated in the pathology of other conditions including diabetes and it has been proposed that oxidative damage to CERU may be the trigger for its dissociation from copper (Shukla et al. 2006). Given that free copper can contribute to the generation of reactive oxygen species, the effect of oxidative damage on CERU may be self-perpetuating and also contribute to oxidative damage of other molecules. Copper binding is integral to the structure of CERU and the loss of copper leads to unfolding of the protein (Sedlák et al. 2008). Furthermore, CERU is found deposited in tissues in a number of diseases including macular degeneration (Rodrigues 2007), preeclampsia (Guller et al. 2008) and atherosclerosis (Hollander et al. 1979). It is currently unknown what effect α_2M or other extracellular chaperones may have on the release of copper from misfolded CERU; however, if unfolding of CERU involves the sequential

release of copper as has been suggested (Sedlák and Wittung-Stafshede 2007), it is possible that extracellular chaperones may interact with partially unfolded intermediate forms of CERU and prevent further unfolding and the subsequent release of copper.

The ability of FGN to undergo extensive conformational changes is essential for its role in blood clotting; however, FGN is known to be particularly sensitive to misfolding as a result of oxidative stress (Shacter et al. 1994) and has comparatively poor thermal stability compared to other blood proteins (Raeker and Johnson 1995). FGN is routinely deposited at sites of tissue injury and inflammation, and its deposition has been implicated in a large number of disease states including age-related macular degeneration (Hageman et al. 2001; Shiose et al. 2004; van der Schaft et al. 1993), cancer (Costantini et al. 1991a, b, 1992; Wojtukiewicz et al. 1989a, 1989b), renal disease (Koffler and Paronetto 1966), systemic lupus erythematosus (Dujovne et al. 1972), atherosclerosis (Shainoff and Page 1972; Sadoshima and Tanaka 1979) and AD (Paul et al. 1999). Although the role of deposited FGN in these diseases is unclear, the pro-inflammatory responses by platelets and immune cells that are induced by FGN are reportedly enhanced by oxidation, exposure to stress hormones or nanoparticle-induced unfolding of the protein (Barbucci et al. 2003, 2007; Deng et al. 2011; Upchurch et al. 1998; Azizova et al. 2007); thus, FGN misfolding may be an important factor in the pathology of many diseases.

AGP, also known as orosomucoid, is a protein for which the precise biological function is unknown; however, numerous immunomodulatory activities that appear to be dependent on its glycosylation have been described (Fournier et al. 2000). It has also been reported that AGP can act as a molecular chaperone (Zsila 2010); therefore, one possibility is that the interaction of AGP with α_2M observed in this study may have been via shared affinity for misfolded client proteins. However, it should be noted that reports of AGP chaperone activity are currently limited to a single study and its ability to act as a chaperone has not been reproducible in our hands. Interestingly, in the ZAC fractions from both control and stressed plasma, the majority of AGP detected in Western blots was present as species larger than that expected for the most abundant form of the protein (43 kDa). It is currently unknown exactly what these species are; however, it is possible that they are hyperglycosylated forms of AGP. Highly glycosylated forms of AGP are increased in numerous pathological states including rheumatoid arthritis (Elliott et al. 1997), septic shock (Brinkman-van der Linden et al. 1996) and myocardial infarction (Poland et al. 2005). Furthermore, there is some evidence that highly glycosylated forms of AGP are susceptible to deposition (Poland et al. 2005). Although hyperglycosylation of protein is generally considered to be stabilizing (Ceaglio et al. 2010), it is known to be destabilizing in some cases (Lee et al. 2010). Hyperglycosylated AGP, which is present in normal plasma at low levels (Poland et al. 2005; Theilgaard-Mönch et al. 2005), may have a decreased stability and therefore preferentially interact with α_2M; however, further work is needed to confirm whether this is the case.

C3 is an inherently unstable protein that converts to C3b by spontaneous hydrolysis of the thioester bond (Pangburn et al. 1981). Furthermore, complement activation may occur in vitro even in the presence of protease inhibitors generating further cleavage products from C3b, including iC3b, C3c and C3d (Pfeifer et al. 1999; Momeni et al. 2012). Following hydrolysis to form C3b and subsequent cleavage to yield the inhibited form iC3b, C3 undergoes major conformational changes including progressive unfolding, although the precise nature of these changes is debated (Janssen et al. 2007; Ajees et al. 2007). There are no known reports of native C3 interacting with α_2M, and the results of this study suggest that α_2M interacts only with modified forms of C3. Increased levels of C3 breakdown products are found in the blood of patients with many diseases including systemic lupus erythematosus (Negoro et al. 1989), arthritis (Kanayama et al. 1986) and age-related macular degeneration (Scholl et al. 2008). It has been suggested that overproduction of C3b/iC3b may exacerbate neuronal disease by priming microglia through interaction with complement receptor 3 (CR3; also known as MAC-1 (Ramaglia et al. 2012)). Interestingly, CR3 has been identified as one of a number of innate immune system receptors (also including scavenger receptors and toll-like receptors) that are stimulated by misfolded proteins including denatured FGN (Jozefowski and Marcinkiewicz 2010; Deng et al. 2011).

The discovery of major endogenous plasma clients for α_2M is an important step towards characterizing the role(s) of α_2M in extracellular proteostasis. This study identifies that in human plasma exposed to physiologically relevant stress, several abundant acute phase proteins are major endogenous clients for α_2M. Regardless of the normal plasma residency half-lives of these proteins, under disease conditions, they are known to accumulate, particularly when damaged or modified (discussed above). Whether or not the chaperone action of α_2M can influence the pathological activities of these proteins remains to be determined. It has been shown that following activation by proteases, α_2M–misfolded client protein complexes can bind to LRP (French et al. 2008). Thus, it is plausible that the formation of complexes between α_2M and misfolded FGN or C3b/iC3b may direct the latter two species to LRP and thereby prevent their interaction with CR3 and subsequent pro-inflammatory responses. Thus, the anti-inflammatory activity of α_2M which has largely been attributed to its ability to interact with cytokines (Desser et al. 2001; Roma et al. 2010) may also be a consequence of it facilitating the clearance of

immunostimulatory misfolded client proteins. Activation of α_2M (to yield the receptor recognised form) via administration of chemical compounds or proteases is already being explored as a possible therapeutic strategy for the treatment of certain cancers (Lauer et al. 2001) and also a range of inflammatory conditions including pancreatitis (Roma et al. 2010) and arthritis (Desser et al. 2001); these approaches are targeting the ability of α_2M to facilitate the clearance of cytokines. Given the wide range of diseases in which protein misfolding and chronic inflammation are implicated, there is great scope for development of chaperone-based therapies to treat these conditions. The in vivo effects of the chaperone activity of α_2M on pathologies arising from protein misfolding and aggregation, including those pathologies involving the protein clients identified here, justify further studies.

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References

Adler V, Kryukov V (2007) Serum macroglobulin induces prion protein transition. Neurochem J 1:43–52

Ajees AA, Gunasekaran K, Narayana SVL, Murthy HMK (2007) Crystallography: crystallographic evidence for deviating C3b structure (reply). Nature 448:E2–E3

Azizova OA, Aseichev AV, Piryazev AP, Roitman EV, Shcheglovitova ON (2007) Effects of oxidized fibrinogen on the functions of blood cells, blood clotting, and rheology. Bull Exp Biol Med 144(3):397–407

Barbucci R, Lamponi S, Magnani A (2003) Fibrinogen conformation and platelet reactivity in relation to material–blood interaction: effect of stress hormones. Biomacromolecules 4:1506–1513

Barbucci R, Lamponi S, Magnani A (2007) Role of fibrinogen activation in platelet activation. Biomacromolecules 8(2):523–531

Barrett AJ, Starkey PM (1973) The interaction of α2-macroglobulin with proteinases. Characteristics and specificity of the reaction, and a hypothesis concerning its molecular mechanism. Biochem J 133:709–724

Bekard I, Asimakis P, Bertolini J, Dunstan D (2011) The effects of shear flow on protein structure and function. Biopolymers 95:733–745

Borth W (1992) α2-Macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J 6:3345–3353

Brewer GJ, Kanzer SH, Zimmerman EA, Celmins DF, Heckman SM, Dick R (2010) Copper and ceruloplasmin abnormalities in Alzheimer's disease. Am J Alzheimers Dis Other Demen 25 (6):490–497

Brinkman-van der Linden EC, Van Ommen EC, Van Dijk W (1996) Glycosylation of α1-acid glycoprotein in septic shock: changes in degree of branching and in expression of sialyl Lewis (x) groups. Glycoconj J 13(1):27–31

Ceaglio N, Etcheverrigaray M, Kratje R, Oggero M (2010) Influence of carbohydrates on the stability and structure of a hyperglycosylated human interferon alpha mutein. Biochimie 92(8):971–978

Costantini V, Zacharski LR, Memoli VA, Kudryk BJ, Rousseau SM (1991a) Fibrinogen deposition without thrombin generation in primary human breast cancer tissue. Cancer Res 51:349–353

Costantini V, Zacharski LR, Memoli VA, Kudryk BJ, Rousseau SM, Stump DC (1991b) Occurrence of components of fibrinolysis pathways in situ in neoplastic and nonneoplastic human breast tissue. Cancer Res 51:354–358

Costantini V, Zacharski LR, Memoli VA, Kisiel W, Kudryk BJ, Rousseau SM, Stump DC (1992) Fibrinogen deposition and macrophage-associated fibrin formation in malignant and nonmalignant lymphoid tissue. J Lab Clin Med 119:124–131

Cunnion KM, Hair PS, Buescher ES (2004) Cleavage of Complement C3b to iC3b on the surface of *Staphylococcus aureus* is mediated by serum complement factor I. Infect Immun 72:2858–2863

Davis GE (1992) The Mac-1 and p150,95 β2 integrins bind denatured proteins to mediate leukocyte cell-substrate adhesion. Exp Cell Res 200:242–252

Deng ZJ, Liang M, Monteiro M, Toth I, Minchin RF (2011) Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat Nanotechnol 6:39–44

Desser L, Holomanova D, Zavadova E, Pavelka K, Mohr T, Herbacek I (2001) Oral therapy with proteolytic enzymes decreases excessive TGF-b levels in human blood. Cancer Chemother Pharmacol 47: S10–S15

Di Stasio E, De Cristofaro R (2010) The effect of shear stress on protein conformation: physical forces operating on biochemical systems: the case of von Willebrand factor. Biophys Chem 153:1–8

Doan N, Gettins PG (2007) Human α2-macroglobulin is composed of multiple domains, as predicted by homology with complement component C3. Biochem J 407:23–30

Dujovne I, Pollak VE, Pirani CL, Dillard MG (1972) The distribution and character of glomerular deposits in systemic lupus erythematosus. Kidney Int 2:33–50

Elliott MA, Elliott HG, Gallagher K, McGuire J, Field M, Smith KD (1997) Investigation into the concanavalin a reactivity, fucosylation and oligosaccharide microheterogeneity of α1-acid glycoprotein expressed in the sera of rheumatoid arthritis. J Chromatogr B: Biomed Sci Appl 688:229–237

Feldman SR, Ney KA, Gonias SL, Pizzo SV (1983) In vitro binding and in vivo clearance of human α2-macroglobulin after reaction with endoproteases from four different classes. Biochem Biophys Res Commun 114(2):757–762

Fournier T, Medjoubi-N N, Porquet D (2000) Alpha-1-acid glycoprotein. Biochem Biophys Acta 1482:157–171

French K, Yerbury JJ, Wilson MR (2008) Protease activation of α2-macroglobulin modulates a chaperone-like action with broad specificity. Biochemistry 47(4):1176–1185

Gehring MR, Shiels BR, Northemann W, de Bruijn MH, Kan CC, Chain AC, Noonan DJ, Fey GH (1987) Sequence of rat liver α2-macroglobulin and acute phase control of its messenger RNA. J Biol Chem 262(1):446–456

Guller S, Buhimschi CS, Ma YY, Huang ST, Yang L, Kuczynski E, Zambrano E, Lockwood CJ, Buhimschi IA (2008) Placental expression of ceruloplasmin in pregnancies complicated by severe preeclampsia. Lab Investig 88(10):1057–1067

Hageman GS, Luthert PJ, Chong NHV, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20:705–732

Harpel PC (1976) Human α2-macroglobulin. Meth Enzymol 45:639–652

Harpel PC, Hayes MB, Hugli TE (1979) Heat-induced fragmentation of human alpha 2-macroglobulin. J Biol Chem 254:8669–8678

Herczenik E, Bouma B, Korporaal SJA, Strangi R, Zeng Q, Gros P, Van Eck M, Van Berkel TJC, Gebbink MFBG, Akkerman JWN (2007) Activation of human platelets by misfolded proteins. Arterioscler Thromb Vasc Biol 27:1657–1665

Hespanhol MR, Mantovani B (2002) Phagocytosis by macrophages mediated by receptors for denatured proteins—dependence on tyrosine protein kinases. Braz J Med Biol Res 35:383–389

Hollander W, Colombo MA, Kirkpatrick B, Paddock J (1979) Soluble proteins in the human atherosclerotic plaque. With spectral reference to immunoglobulins, C3-complement component, alpha 1-antitrypsin and alpha 2-macroglobulin. Atherosclerosis 34(4):391–405

Housley J (1968) Alpha2-macroglobulin levels in disease in man. J Clin Pathol 21:27–31

Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem 274:6875–6881

Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC (2002) Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia 40 (2):195–205

Imber MJ, Pizzo SV (1981) Clearance and binding of two electrophoretic "fast" forms of human α2-macroglobulin. J Biol Chem 256:8134–8139

Islam KN, Takahashi M, Higashiyama S, Myint T, Uozumi N, Kayanoki Y, Kaneto H, Kosaka H, Taniguchi N (1995) Fragmentation of ceruloplasmin following non-enzymatic glycation reaction. J Biochem 118(5):1054–1060

Jana M, Palencia CA, Pahan K (2008) Fibrillar amyloid-beta peptides activate microglia via TLR2: implications for Alzheimer's disease. J Immunol 181:7254–7262

Janssen BJC, Read RJ, Brunger AT, Gros P (2007) Crystallographic evidence for the deviating structure of C3b? Nature 448:E1–E2

Jozefowski S, Marcinkiewicz J (2010) Aggregates of denatured proteins stimulate nitric oxide and superoxide production in macrophages. Inflamm Res 59:277–289

Kanayama Y, Kurata Y, McMillan R, Tamerius JD, Negoro N, Curd JG (1986) Direct quantitation of activated C3 in human plasma with monoclonal anti-iC3b-C3d-neoantigen. J Immunol Methods 88 (1):33–36

Kaplan J, Ray FA, Keogh EA (1981) Recognition of nucleophile-treated α2-macroglobulin by the alveolar macrophage α2-macroglobulin-protease complex receptor. J Biol Chem 256:7705–7707

Koffler D, Paronetto F (1966) Fibrinogen deposition in acute renal failure. Am J Pathol 49:383–395

Kranenburg O, Bouma B, Kroon-Batenburg LMJ, Reijerkerk A, Wu YP, Voest EE, Gebbink MFBG (2002) Tissue-type plasminogen activator is a multiligand cross-β structure receptor. Curr Biol 12:1833–1839

Kristensen T, Moestrup SK, Gliemann J, Bendtsen L, Sand O, Sottrup-Jensen L (1990) Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the α2-macroglobulin receptor. FEBS Lett 276:151–155

LaMarre J, Wollenberg GK, Gonias SL, Hayes MA (1991) Cytokine binding and clearance properties of proteinase-activated alpha 2-macroglobulins. Lab Investig 65(1):3–14

Larsson LJ, Lindahl P, Hallen-Sandgren C, Bjork I (1987) The conformational changes of α2-macroglobulin induced by methylamine or trypsin. Biochem J 243:47–54

Lauer D, Müller R, Cott C, Otto A, Naumann M, Birkenmeir G (2001) Modulation of growth factor binding properties of α2-macroglobulin by enzyme therapy. Cancer Chemother Pharmacol 47:S4–S9

Lee WC, Kang D, Causevic E, Herdt AR, Eckman EA, Eckman CB (2010) Molecular characterization of mutations that cause globoid cell leukodystrophy and pharmacological rescue using small molecule chemical chaperones. J Neurosci 30(16):5489–5497

Malek AM, Alper SL, Izumo S (1999) Hemodynamic shear stress and its role in atherosclerosis. JAMA 282(21):2035–2042

Marx G (1988) Zinc binding to fibrinogen and fibrin. Arch Biochem Biophys 266(1):285–288

Momeni N, Brudin L, Behnia F, Nordström B, Yosefi-Oudarji A, Sivberg B, Joghataei MT, Persson BL (2012) High complement factor I activity in the plasma of children with autism spectrum disorders. Autism Research and Treatment 2012:1–6

Motomiya Y, Ando Y, Haraoka K, Sun X, Iwamoto H, Uchimura T, Maruyama I (2003) Circulating level of α2-macroglobulin–β2-microglobulin complex in hemodialysis patients. Kidney Int 64:2244–2252

Narita M, Holtzman DM, Schwartz AL, Bu G (1997) α2-Macroglobulin complexes with and mediates the endocytosis of β-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. J Neurochem 69:1904–1911

Negoro N, Okamura M, Takeda T, Koda S, Amatsu K, Inoue T, Curd JG, Kanayama Y (1989) The clinical significance of iC3b neoantigen expression in plasma from patients with systemic lupus erythematosus. Arthritis Rheum 32(10):1233–1242

Ottaviano FG, Handy DE, Loscalzo J (2008) Redox regulation in the extracellular environment. Circ J 72:1–16

Pangburn MK, Schreiber RD, Müller-Eberhard HJ (1981) Formation of the initial C3 convertase of the alternative complement pathway. J Exp Med 154:856–867

Paul J, Strickland S, Melchor JP (1999) Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. J Exp Med 204 (8):1999–2008

Pfeifer PH, Kawahara MS, Huglia TE (1999) Possible mechanism for in vitro complement activation in blood and plasma samples: futhan/EDTA controls in vitro complement activation. Clin Chem 45:1190–1199

Poland DCW, Vallejo JJG, Niessen HWM, Nijmeyer R, Calafat J, Hack CE, Van het Hof B, Van Dijk W (2005) Activated human PMN synthesize and release a strongly fucosylated glycoform of α1-acid glycoprotein, which is transiently deposited in human myocardial infarction. J Leukoc Biol 78:453–461

Raeker M, Johnson LA (1995) Thermal and functional properties of bovine blood plasma and egg white proteins. J Food Sci 60 (4):685–690

Ramaglia V, Hughes TR, Donev RM, Ruseva MM, Wu X, Huitinga I, Baas F, Neal JW, Morgan BP (2012) C3-dependent mechanism of microglial priming relevant to multiple sclerosis. Proc Natl Acad Sci U S A 109(3):965–970

Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY (2004) Reference distributions for α2-macroglobulin: a practical, simple and clinically relevant approach in a large cohort. J Clin Lab Anal 18:139–147

Rodrigues EB (2007) Inflammation in dry age-related macular degeneration. Ophthalmologica 221(3):143–152

Roma SM, Di Loreto VE, Rigalli A (2010) Effect of the treatment with monofluorophosphate on the survival and tissue damage in rats with pancreatitis. Acta Gastroenterol Latinoam 41:122–127

Rothbard JB, Kurnellas MP, Brownell S, Adams CM, Su L, Axtell RC, Chen R, Fathman CG, Robinson WH, Steinman L (2012) Therapeutic effects of systemic administration of chaperone αB-crystallin associated with binding proinflammatory plasma proteins. J Biol Chem 287:9708–9721

Sadoshima S, Tanaka K (1979) Fibrinogen and low density lipoprotein in the development of cerebral atherosclerosis. Atherosclerosis 34 (2):93–103

Sato M, Schilsky ML, Stockert RJ, Morell AG, Sternlieb I (1990) Detection of multiple forms of human ceruloplasmin. A novel 200,000 form. J Biol Chem 265(5):2533–2537

Scholl HPN, Charbel Issa P, Walier M, Janzer S, Pollok-Kopp B, Börncke F, Fritsche LG, Chong NV, Fimmers R, Weinker T, Holz FG, Weber BHF, Oppermann M (2008) Systemic complement activation in age-related macular degeneration. PLoS One 3(7): e2593

Scorza G, Minetti M (1998) One-electron oxidation pathway of thiols by peroxynitrite in biological fluids: bicarbonate and ascorbate promote the formation of albumin disulphide dimers in human blood plasma. Biochem J 329:405–413

Scott BJ, Bradwell AR (1983) Identification of serum binding proteins for iron, zinc, cadmium, nickel and calcium. Clin Chem 29 (4):629–633

Sedlák E, Wittung-Stafshede P (2007) Discrete roles of copper ions in chemical unfolding of human ceruloplasmin. Biochemistry 46 (33):9638–9644

Sedlák E, Žoldák G, Wittung-Stafshede P (2008) Role of copper in thermal stability of human ceruloplasmin. Biophys J 94(4):1384–1391

Shacter E, Williams JA, Lim M, Levine RL (1994) Differential susceptibility of plasma proteins to oxidative modification: examination by Western blot immunoassay. Free Radic Biol Med 17 (5):429–437

Shainoff JR, Page IH (1972) Deposition of modified fibrinogen in the aortic intima. Atherosclerosis 16(3):287–305

Shiose S, Hata Y, Noda Y, Sassa Y, Takeda A, Yoshikawa H, Fujisawa K, Kubota T, Ishibashi T (2004) Fibrinogen stimulates angiogenesis by choroidal endothelial cells via autocrine VEGF. Graefes Arch Clin Exp Ophthalmol 242:777–783

Shukla N, Maher J, Masters J, Angelini GD, Jeremy JY (2006) Does oxidative stress change ceruloplasmin from a protective to a vasopathic factor? Atherosclerosis 187(2):238–250

Sim RB, Sim E (1981) Autolytic fragmentation of complement components C3 and C4 under denaturing conditions, a property shared with alpha2-macroglobulin. Biochem J 193:129–141

Sottrup-Jensen L (1989) Alpha-macroglobulins: structure shape and mechanism of proteinase complex formation. J Biol Chem 264:11539–11542

Sottrup-Jensen L, Hansen HF (1982) Nascent α2-macroglobulin-trypsin complex: incorporation of amines and water at the thiol esterified Glx residues of alpha-2-macroglobulin during activation with trypsin. Biochem Biophys Res Commun 107:93–100

Sottrup-Jensen L, Stepanik TM, Kristensen T, Wierzbicki DM, Jones CM, Lonblad PB, Magnusson S, Petersen TE (1984) Primary structure of α2-macroglobulin. J Biol Chem 259(10):8318–8327

Sottrup-Jensen L, Gliemann J, Van Leuven F (1986) Domain structure of human α2-macroglobulin. Characterization of a receptor-binding domain obtained by digestion with papain. J Biol Chem 261:20–24

Squitti R, Barbati G, Rossi L, Ventriglia M, Dal Forno G, Cesaretti S, Moffa F, Cardi I, Cassetta E, Pasqualletti P, Calabrese L, Lupoi D, Rossini PM (2006) Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 67(1):76–82

Squitti R, Quattrocchi CC, Dal Forno G, Antuono P, Wekstein DR, Capo CR, Salustri C, Rossini PM (2008) Ceruloplasmin (2-D PAGE) pattern and copper content in serum and brain of Alzheimer's disease patients. Biomark Insights 1:205–213

Squitti R, Bressi F, Pasqualletti P, Bonomini C, Ghidoni R, Binetti G, Cassetta E, Moffa F, Ventriglia M, Vernieri F, Rossini PM (2009) Longitudinal prognostic value of serum "free" copper in patients with Alzheimer's disease. Neurology 72(1):50–55

Theilgaard-Mönch K, Jacobsen LC, Rasmussen T, Niemann CU, Udby L, Borup R, Gharib M, Arkwright PD, Gombart AF, Calafat J, Porse T, Borregaard N (2005) Highly glycosylated α1-acid glycoprotein is synthesized in myelocytes, stored in secondary granules, and released by activated neutrophils. J Leukoc Biol 78 (2):462–470

Udan MLD, Ajit D, Crouse NR, Nichols MR (2008) Toll-like receptors 2 and 4 mediate Aβ(1–42) activation of the innate immune response in a human monocytic cell line. J Neurochem 104:524–533

Upchurch GR, Ramdev N, Walsh MT, Loscalzo J (1998) Prothombotic consequences of the oxidation of fibrinogen and their inhibition by aspirin. J Thromb Thrombolysis 5:9–14

van der Schaft TL, Mooy CM, de Bruijn WC, de Jong PT (1993) Early stages of age-related macular degeneration: an immunofluorescence and electron microscopy study. Br J Ophthalmol 77:657–661

van Westrhenen R, Westra WM, van den Born J, Krediet RT, Keuning ED, Hiralall J, Dragt C, Hekking HP (2006) Alpha-2-macroglobulin and albumin are useful serum proteins to detect subclinical peritonitis in the rat. Perit Dial Int 26:101–107

Wojtukiewicz MZ, Zacharski LR, Memoli VA, Kisiel W, Kudryk BJ, Rousseau SM, Stump DC (1989a) Absence of components of coagulation and fibrinolysis pathways in situ in mesothelioma. Thromb Res 55:279–284

Wojtukiewicz MZ, Zacharski LR, Memoli VA, Kisiel W, Kudryk BJ, Rousseau SM, Stump DC (1989b) Indirect activation of blood coagulation in colon cancer. J Thromb Haemost 62:1062–1066

Wyatt AR, Wilson MR (2010) Identification of human plasma proteins as major clients for the extracellular chaperone clusterin. J Biol Chem 285:3532–3539

Wyatt AR, Yerbury JJ, Wilson MR (2009) Structural characterization of clusterin–client protein complexes. J Biol Chem 284:21920–21927

Yerbury JJ, Rybchyn MS, Easterbrook-Smith SB, Henriques C, Wilson MR (2005) The acute phase protein haptoglobin is a mammalian extracellular chaperone with an action similar to clusterin. Biochemistry 44:10914–10925

Yerbury JJ, Kumita JR, Meehan S, Dobson CM, Wilson MR (2009) α2-Macroglobulin and haptoglobin suppress amyloid formation by interacting with prefibrillar protein species. J Biol Chem 284:4246–4252

Zsila F (2010) Chaperone-like activity of the acute-phase component human serum α1-acid glycoprotein: inhibition of thermal- and chemical-induced aggregation of various proteins. Bioorg Med Chem Lett 20(3):1205–1209