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Abstract

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Keywords

human, macroglobulin, 2, action, plasma, chaperone, acute, clients, major, proteins, phase, CMMB

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Acute Phase Proteins Are Major Clients For the Chaperone Action of α_2 -Macroglobulin in Human Plasma

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Keywords: α_2 -macroglobulin, chaperone, protein misfolding, acute-phase proteins

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_2 M) 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_2 M after plasma was stressed. Furthermore, the formation of complexes between α_2 M 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_2 M plays an important role in extracellular proteostasis by sequestering misfolded proteins and targeting them for disposal, particularly during acute phase reactions.

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 is a characteristic of many highly debilitating age-related diseases including arthritis, macular degeneration, type II diabetes and Alzheimer's disease. 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 oxidizing 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 recognize 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; Hespagnol 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_2 M) 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_2 M 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_2 M is a large homotetrameric glycoprotein (720 kDa) that is 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_2 M 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_2 M, including a series of eight fibronectin type-3 macroglobulin domains (the last also being a receptor binding domain, RBD), an α -helical TED (thioester containing domain), and a CUB (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_2 M 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_2 M 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_2 M generating a covalent linkage (Sottrup-Jensen and Hansen 1982). Similar conformational changes in α_2 M may be brought about by nucleophile attack of the thiol ester groups alone (Larsson et al. 1987). Transformation of α_2 M by nucleophilic attack of the thiol ester results in the exposure of a cryptic binding site on each α_2 M 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_2 M facilitates the extracellular

clearance of proteases (Feldman et al. 1983) and other non-covalently bound ligands, including cytokines (LaMarre et al. 1991).

$\alpha_2\text{M}$ 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 $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$ may play an important role in the clearance of extracellular misfolded proteins (French et al. 2008; Yerbury et al. 2009). $\alpha_2\text{M}$ is abundant in most extracellular fluids including blood plasma where it is present at between 1.5-2.5 mg/ml in adult humans (Ritchie et al. 2004). Similar to other members of the extracellular chaperone family, depletion of $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$. 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 $\alpha_2\text{M}$ 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 dyne/cm²) 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 1-6 dyne/cm² in the venous system and 10-70 dyne/cm² 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 Zn²⁺ 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 $\alpha_2\text{M}$ (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 $\alpha_2\text{M}$. The amount of lower molecular mass protein present was similar to the control, however, the proportion of higher molecular mass proteins detected was about 3 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 $\alpha_2\text{M}$ alone.

Reducing SDS-PAGE analysis of ZAC-purified proteins from control plasma showed that it mostly contained protein corresponding to the mass of reduced $\alpha_2\text{M}$ subunits (180 kDa), together with a small amount of protein having an apparent mass corresponding to that of the fragments formed when $\alpha_2\text{M}$ subunits are cleaved by proteases approximately in half at the bait region (85 kDa)(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 kDa and the 85 kDa species purified by ZAC from control or stressed plasma were specifically detected by anti- $\alpha_2\text{M}$ antibodies (Fig. 1d). Bands at 120 kDa and 60 kDa were also specifically detected by anti- $\alpha_2\text{M}$ antibodies and correspond to the expected size of fragments of $\alpha_2\text{M}$ that are known to be generated by heating (Harpel et al. 1979). Two additional bands close to 50 kDa were also detected by anti- $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$ 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 $\alpha_2\text{M}$.

Western blot analysis was carried out for 13 abundant plasma proteins to determine the relative amounts that copurified with $\alpha_2\text{M}$ 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 115kDa; (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 kDa, 56 kDa 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, 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 kDa 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 kDa 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_2 M and not merely present as an artefact of ZAC, sandwich ELISA was used to detect α_2 M-client protein complexes. On plates coated with anti- α_2 M 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_2 M 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_2 M expression is markedly increased during the acute phase in rats (Gehring et al. 1987), plasma concentrations of α_2 M do not increase during the acute phase response in humans (Harpel 1976; Housley 1968). However, the plasma concentration of α_2 M in healthy individuals is ~ 1000-fold higher in humans compared to rats (1.5-2.5 mg/ml in humans (Ritchie et al. 2004) versus 12.7-18.4 μ g/ml in rats (van Westrhenen et al. 2006)). Therefore, increasing the plasma concentration of α_2 M 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_2 M were uniquely detected in the sample prepared from stressed plasma; these unique bands represent proteins that only co-purify with α_2 M from stressed plasma. The majority of protein isolated from stressed plasma by ZAC eluted close to the expected mass of tetrameric α_2 M. This is consistent with previous reports that α_2 M forms complexes with misfolded client proteins that are indistinguishable from α_2 M alone by SuperoseTM 6 SEC (French et al. 2008) and suggests that the capacity of α_2 M 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 $\geq 4 \times 10^4$ kDa) were approximately 3-fold 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_2 M and client proteins in response to stress.

α_2 M 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_2 M 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_2 M-client protein complexes are rapidly cleared from the bloodstream. If this mechanism were to fail or become overwhelmed (simulated by incubating plasma in the absence of cells), only then would α_2 M-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_2 M; SDS-PAGE analysis supports that clients for α_2 M 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_2 M 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_2 M. It was recently shown that when α B-crystallin (an intracellular chaperone with “holdase”-type activity similar to α_2 M 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 α B-client proteins identified in (Rothbard et al. 2012) included all four of the α_2 M 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 Alzheimer’s disease (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; Squitti et al. 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_2 M 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; Costantini et al. 1991b; Wojtukiewicz et al. 1989a; Wojtukiewicz et al. 1989b; Costantini et al. 1992), 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 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 destabilising 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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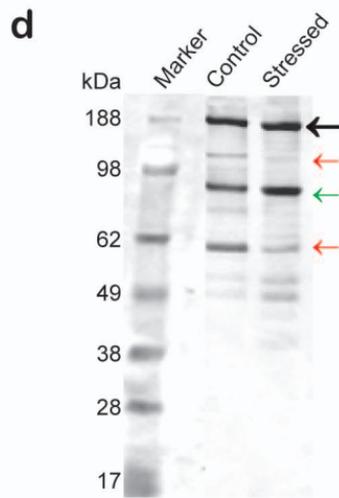
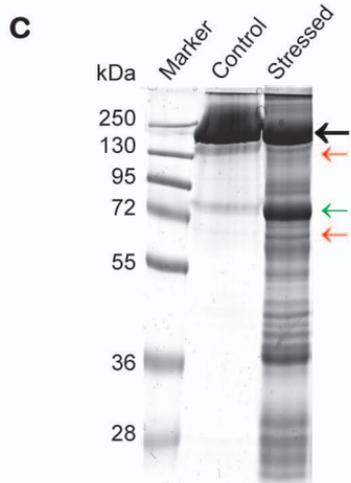
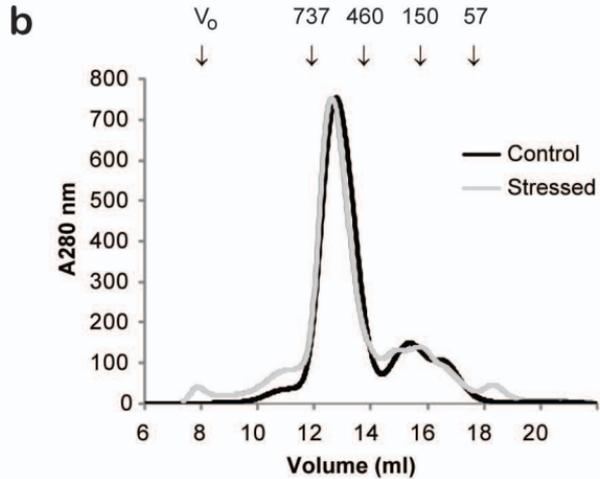
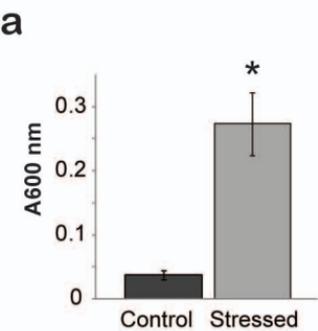
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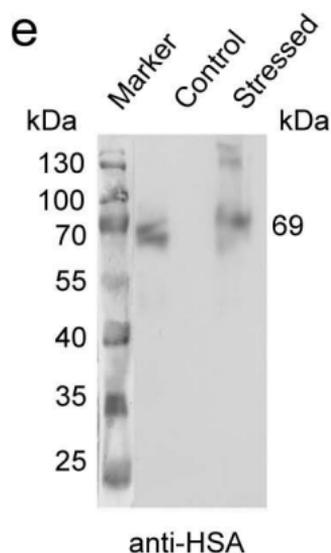
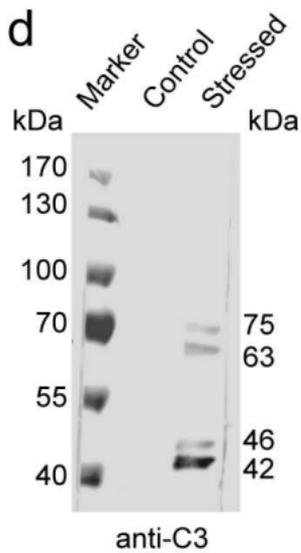
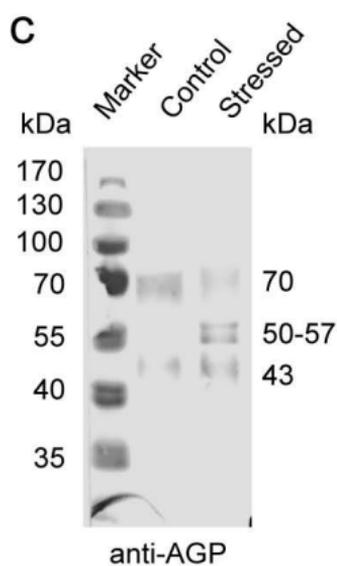
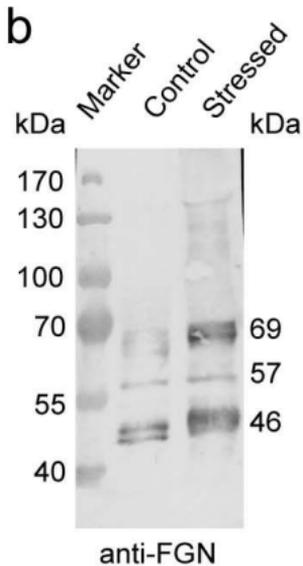
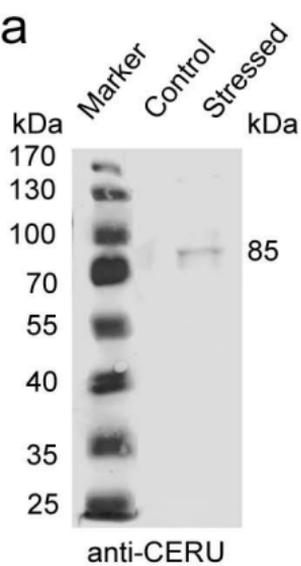
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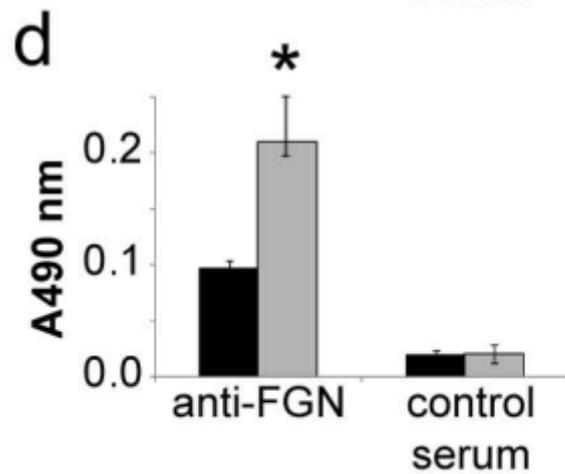
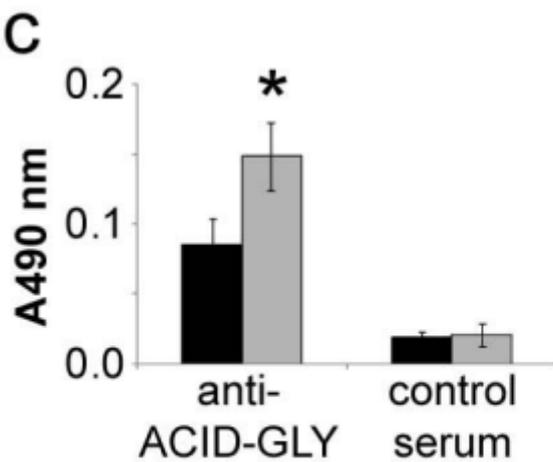
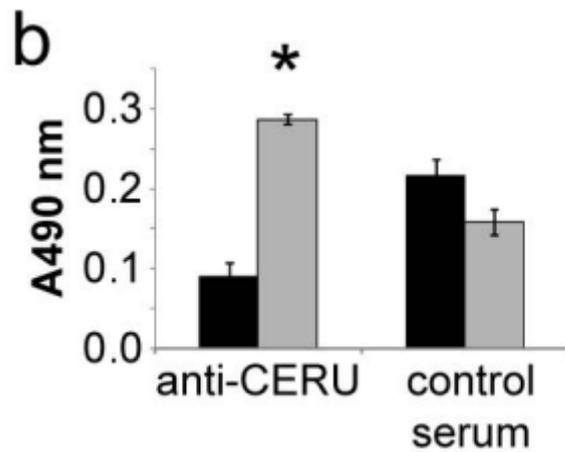
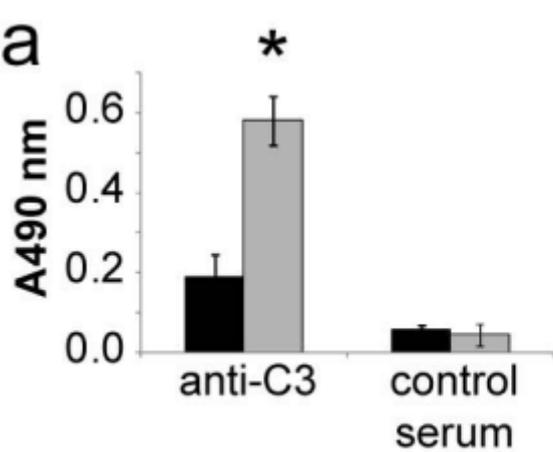
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Online Resource 1. Details of the primary antibodies/antisera, secondary antibodies and control isotype/species-matched antibodies/sera used in this study including their manufacturer, type and applications.

Species reactivity	Antigen	Host Species	Type	Manufacturer	Applications
Primary antibodies/antisera					
Human	Albumin	Rabbit	Antiserum	Sigma-Aldrich	Western blot
Human	IgG	Mouse	Polyclonal IgG	BD Pharmingen	Western blot
Human	Transferrin	Goat	Antiserum	Sigma-Aldrich	Western blot
Human	Fibrinogen	Goat	Antiserum	Sigma-Aldrich	Western blot, ELISA
Human	IgA	Goat	Polyclonal IgG-HRP conjugate	Bio-RAD	Western blot
Human	IgM	Goat	Polyclonal IgG-HRP conjugate	Bio-RAD	Western blot
Human	α 1-antitrypsin	Rabbit	Polyclonal IgG	Sigma-Aldrich	Western blot
Human	Haptoglobin	Rabbit	Polyclonal IgG	Sigma-Aldrich	Western blot
Human	Ceruloplasmin	Goat	Antiserum	Sigma-Aldrich	Western blot, ELISA
Human	Apolipoprotein A1	Sheep	Polyclonal IgG	AbD Serotec	Western blot
Human	Complement component C3	Goat	Antiserum	AbD Serotec	Western blot, ELISA
Human	α 1-acid glycoprotein	Goat	Antiserum	Sigma-Aldrich	Western blot, ELISA
Human	Clusterin	Mouse	Monoclonal IgG	(Murphy et al. 1988)	Western blot
Human	Histidine-rich glycoprotein	Goat	Polyclonal IgG	AbD Serotec	Western blot
Human	α 2-macroglobulin	Rabbit	Polyclonal IgG	DakoCytomation	Western blot
Human	α 2-macroglobulin	Chicken	Affinity purified polyclonal IgY	Abcam	ELISA (coating)
Secondary Antibodies					
Goat/sheep	IgG	Mouse	Monoclonal IgG-HRP conjugate	Sigma-Aldrich	Western blot, ELISA
Rabbit	IgG	Sheep	Polyclonal IgG-HRP conjugate	Millipore	Western blot
Mouse	IgG	Sheep	Polyclonal IgG-HRP conjugate	Millipore	Western blot
Species/isotype-matched control antibodies/sera					
N/A	None	Goat	Serum	Sigma-Aldrich	ELISA
N/A	None	Chicken	Purified IgY	Sigma-Aldrich	ELISA (coating)

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