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Review

Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles

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Abstract

Serine proteinase inhibitors (Serpins) are irreversible suicide inhibitors of proteases that regulate diverse physiological processes such as coagulation, fibrinolysis, complement activation, angiogenesis, apoptosis, inflammation, neoplasia and viral pathogenesis. The molecular structure and physical properties of serpins permit these proteins to adopt a number of variant conformations under physiological conditions including the native inhibitory form and several inactive, non-inhibitory forms, such as complexes with protease or other ligands, cleaved, polymerised and oxidised. Alterations of a serpin which affect its structure and/or secretion and thus reduce its functional levels may result in pathology. Serpin dysfunction has been implicated in thrombosis, emphysema, liver cirrhosis, immune hypersensitivity and mental disorders. The loss of inhibitory activity of serpins necessarily results in an imbalance between proteases and their inhibitors, but it may also have other physiological effects through the generation of abnormal concentrations of modified, non-inhibitory forms of serpins. Although these forms of inhibitory serpins are detected in tissues and fluids recovered from inflammatory sites, the important questions of which conditions result in generation of different molecular forms of serpins, what biological function these forms have, and which of them are directly linked to pathologies and/or may be useful markers for characterisation of disease states, remain to be answered. Elucidation of the biological activities of non-inhibitory forms of serpins may provide useful insights into the pathogenesis of diseases and suggest new therapeutic strategies.

Keywords: Serpin; Conformational polymorphism; α_1-Antitrypsin; α_1-Antichymotrypsin

1. The serpin superfamily

The regulation of proteolytic enzymes in tissues by endogenous inhibitors is a prerequisite for the maintenance of homeostasis. The proteinase inhibitory activity of human plasma was recognised in 1894 by Fermi and Pernossi [1]. The main inhibitor responsible for antiproteolytic activity was first isolated in 1955 by Shultze and named α_1-antitrypsin (AAT) because of its ability to inhibit trypsin. Using electrophoretic techniques, Laurell and Eriksson 1963 first demonstrated the absence of the AAT fraction in a number of patients' sera, and showed a relationship between AAT deficiency and chronic degenerative lung disease [2]. Identification of the serpin family of proteins occurred with the recognition that ovalbumin, AAT and antithrombin share a 30–50% sequence homology [3,4]. Genetic characterisation has revealed a large group of genes belonging to the serpin family that probably arose by gene duplication of an ancestral gene [5]. The term serpin was later introduced as a general descriptor by Carrell and

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Travis in 1985 for a superfamily of serine proteinase inhibitors in mammalian blood plasma [6]. Overall, the serpins have an average of about 30% sequence identity, increasing to 70% when comparison is limited to hydrophobic sequences. Thus, there has been strong conservation of the internal residues of the serpins, indicating conservation of their tertiary structure. This has been confirmed in the now large number of serpins whose crystal structures have been determined.

There are now over 60 members of the serpin family occurring widely in higher organisms, and also in viruses, insects and plants, but not in bacteria and yeast. The primary function of most of the serpins is the regulation of proteolytic enzymes under both physiological and pathological conditions. On the basis of strong sequence similarities, a number of proteins with no known inhibitory activity have been classified as serpins. For example, thyroxine binding globulin (TBG) and corticosteroid binding globulin (CBG) serve as transporters of lipophilic hormones [7–9], angiotensinogen is a peptide hormone precursor [10], and the more recently described 'orphan' ovalbumin serpins have been implicated in the regulation of cell death [11].

The rapid expansion of the serpin family has permitted further differentiation into subfamilies based on amino acid sequence. For instance, the ovalbumin-type serpin subfamily, which originally included five serpins (chicken ovalbumin, plasminogen activator inhibitor (PAI)-2, the squamous cell carcinoma antigen (SCCA) and leucocyte elastase inhibitor), now has more than 20 members [12]. More recently identified members of the human ovalbumin serpins include a tumour suppressor and angiogenesis inhibitor called maspin [13,14], a bone marrow-associated inhibitory serpin, bomapin [15] and cytoplasmic antiproteinase, also known as proteinase 6 [16]. Myxoma virus expresses SERP-1, the only known virus-encoded serpin that is secreted from infected cells and has anti-inflammatory activity although the specific protease target for SERP-1 that is responsible for its activity is not known yet [17,18]. Thus, a major challenge in serpin biology today is not only finding target proteinases for newly discovered serpins, but also identification of the many existing and newly discovered serpins with functions other than proteinase inhibition.

2. Structural-functional characteristics of serpins

The irreversibility of proteinase inhibition achieved by the serpins has made them the principal inhibitors controlling both intra- and extracellular proteolytic pathways. Serpins regulate such diverse physiological processes as coagulation, fibrinolysis, complement activation, angiogenesis, apoptosis, inflammation, neoplasia and viral pathogenesis. However, their precise mechanism of action in many of these processes remains controversial. The primary function of most members of the serpin family is to neutralise overexpressed serine proteinase activity [19]. Therefore, alterations of a serpin which affect its structure and/or secretion and thereby reduce its functional levels, may result in pathology. Serpin dysfunction has been implicated in thrombosis, emphysema, cirrhosis, immune hypersensitivity, mental disorders and in diseases characterised by connective and other tissue self-destruction [20].

Understanding the lost and altered activities of these dysfunctional serpins has been greatly advanced by correlation of molecular structure studies with analysis of the interaction mechanisms of serpins and their target proteinases, and of their physical properties. Serpins are irreversible, suicide inhibitors of proteinases with a positionally conserved reactive site that acts as a 'bait' for a serine or cysteine proteinase [21]. The X-ray crystal structures of serpins have shown that they are globular proteins with nine α-helices and three β-sheets. The reactive centre loop is localised in an approx. 20 amino acid long peptide segment on the molecular surface. It is linked C-terminally to strand 1 of β-sheet C and N-terminally to strand 5 of β-sheet A (Fig. 1). Proteolytic cleavage of the reactive centre loop by proteinase leads to insertion of the strand N-terminal to the cleavage site as strand 4 in β-sheet A. This unique mechanism of inhibition involves a profound change to a conformation with a higher thermodynamic stability, although the nature and significance of this change has been controversial. The recently reported crystal structure of an α_1-antitrypsin and trypsin complex shows that the reaction of the protease with the reactive centre of the serpin results in cleavage the reactive centre of the serpin, and migration of the proteinase to the opposite pole of the serpin. This linkage of the two molecules causes

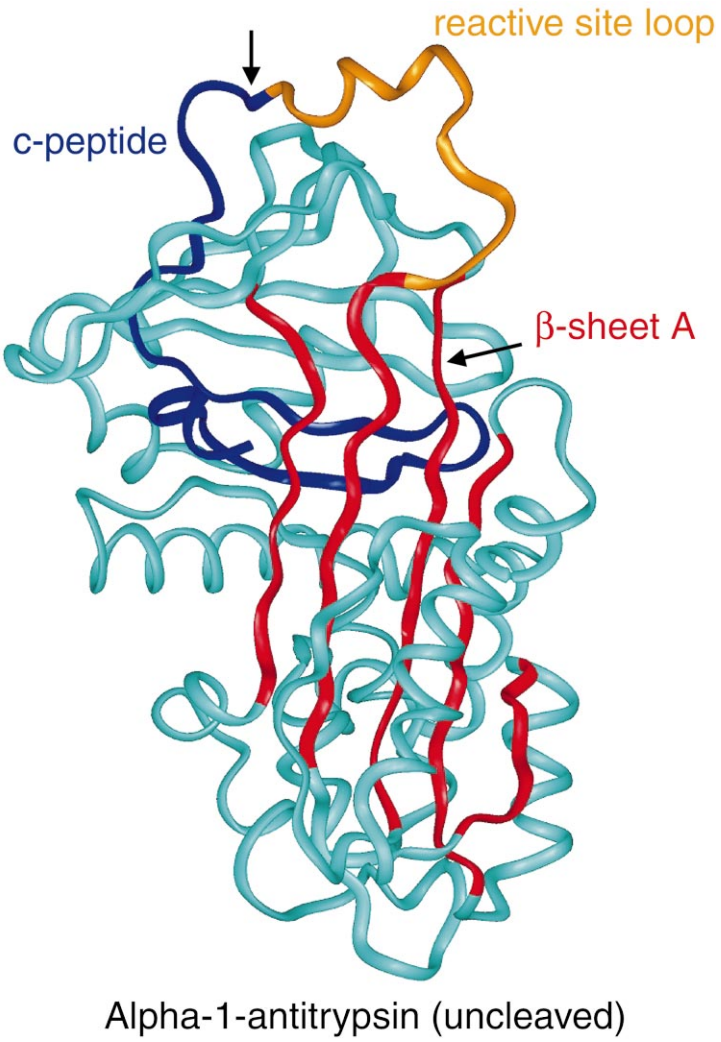

Fig. 1. Schematic picture of the native α_1-antitrypsin. The reactive centre (yellow), β-sheet A (red) and C-terminal peptide (dark blue) are shown in relation to the rest of the structure (light blue).

a large loss of structure in the proteinase [22] induced by serpin-imposed disruption of the active site and of stabilising intramolecular interactions in the protease. Earlier studies had found an increase in proteolytic vulnerability of proteinases in complex with serpins [23–25]. The increased proteolytic lability of the complex results in localised destruction of the proteinase before the slower receptor-mediated uptake from the circulation occurs. Thus, previous studies on a variety of serpin-proteinase complexes and the recently resolved structure of a serpin-proteinase complex explain the unique ability of the serpins to inhibit their cognate proteinases by induced destabilisation, and further confirm the biological success of the serpins as the predominant family of serine proteinases in higher animals.

As mentioned above, serpins possess two structural elements that are conformationally labile and are essential for efficient proteinase inhibition: a reactive centre loop and a β-sheet (β-sheet A) (Fig. 1), which is able to open and accommodate the reactive centre loop after its cleavage by the attacking proteases [26,27]. The conformational mobility of these regions also permits serpins to adopt a number of variant conformations and assembly states under physiological conditions, and renders them sensitive to mutations, of which there are many that result in changes in structure, biosynthesis and/or functional activity. Many of these mutants are linked to specific diseases,

such as the mutants of antithrombin which are associated with thrombosis [28], AAT mutants with lung emphysema and liver diseases [29], and the mutation in the neuroserpin that results in the polymerisation and aggregation of the protein which is associated with familial encephalopathy [30].

Serpin inhibitory activity is also regulated by cofactors which are needed to expose or maintain the reactive centre loop of some serpins. The best example of this phenomenon is antithrombin, which is activated by heparin through induced and transmitted conformational changes that stabilise the proteinase-sensitive active site [31]. Another serpin, PAI-1, which normally exists in a latent form, can be maintained in its functional form in the presence of plasma vitronectin [32]. As well as stabilising PAI-1 in the active conformation, vitronectin also alters the specificity of PAI-1, making it an efficient inhibitor of thrombin. The finding that active PAI-1 specifically inhibits integrin attachment to vitronectin [33] further illustrates the unique functional interdependence that exists between PAI-1 and vitronectin.

Some serpins have been found to have surprising biological activities beyond that of protease inhibition. Nitrosylation of AAT by nitric oxide under inflammatory conditions generates a chemically modified AAT with unexpected cysteine protease inhibitor and antibacterial activities [34]. Another serpin, α_1-antichymotrypsin (ACT), which is primarily an inhibitor of cathepsin G and chymase, has been shown to inhibit purified DNA polymerase [35] and DNA primase [36], possibly through its strong affin-

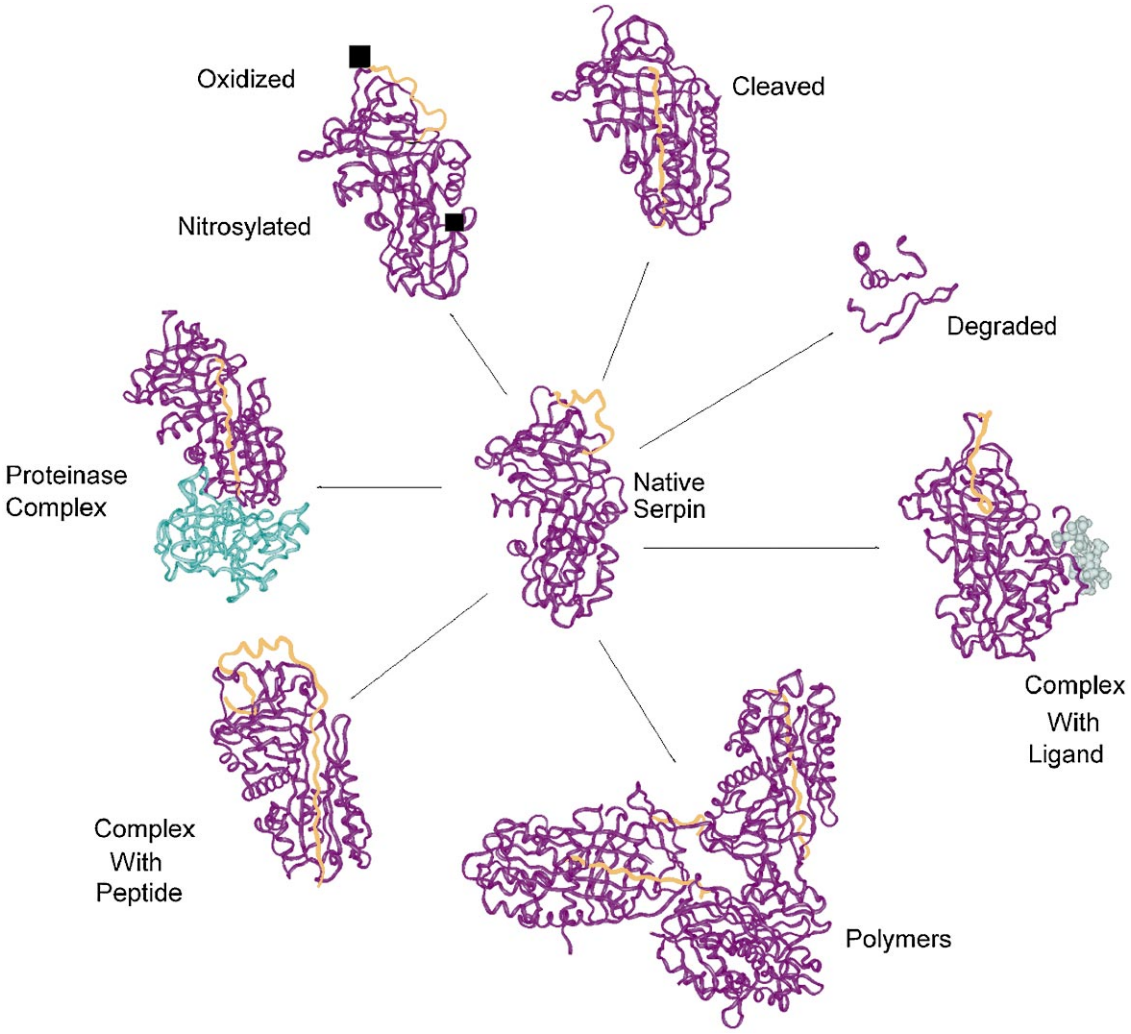

Fig. 2. Conformational polymorphism of inhibitory serpins. Schematic picture of the serpin (lilac) and reactive centre (yellow).

ity for DNA observed in vitro. Native AAT was shown to increase insulin-induced mitogenesis in various fibroblast and epithelial cell lines [37], as well as to inhibit cell growth in human plasma [38]. This latter activity is also observed in three other serpins, ACT, C1-esterase inhibitor and α_2-antiplasmin [38]. Clearly, serpins have multiple biological activities in addition to their role as proteinase inhibitors.

Studies of the functional and conformational polymorphism of inhibitory serpins show that under certain physiological conditions serpins can undergo conformational change due to mutation, chemical modification, or to interaction with other molecular species. Multiple forms of inhibitory serpins have been identified in biological fluids: native inhibitory, inactivated or latent forms, and non-inhibitor forms such as cleaved, polymerised and oxidised (Fig. 2). Inactivation of inhibitors is known to be mediated by several mechanisms: through complex formation with target protease [39], non-specific cleavage by metalloproteases [40], oxidation of the active site methionine [41], and by polymerisation [20]. The loss of inhibitory activity of serpins necessarily results in an imbalance between proteases and their inhibitors, but it may also have other physiological effects through the generation of abnormal concentrations of conformationally modified non-inhibitory forms. For example, it has recently been shown that antithrombin, which functions as an inhibitor of thrombin and other enzymes, in its cleaved conformation has potent antiangiogenic and antitumour activity in mouse models [42]. On the other hand, the degradation of antithrombin by tumours could explain the hypercoagulable state observed with many cancers [43]. Finally the generation of cleaved antithrombin provides additional evidence that plasma proteins leaking from tumour vessels not only form a neostroma for tumour growth but also mobilise angiogenesis inhibitors such as cleaved antithrombin and angiostatin generated from plasminogen [44].

The recognition that multiple functions and pathologies can arise from increased levels of different polymorphs of the same serpin is a relatively novel concept. Some inhibitory serpins, like α_1-antitrypsin and α_1-antichymotrypsin, are well studied examples of these phenomena and illustrate how single serpins can play multiple roles in systemic disorders.

3. Non-inhibitory forms of α_1-antitrypsin and their role in systemic disorders

AAT is an important component of the serine proteinase inhibitor system in humans, with the physiological function of inhibiting target proteinases such as neutrophil elastase and proteinase 3. It also inhibits other serine proteinases, including cathepsin G, thrombin, trypsin and chymotrypsin, but with lower efficiency than that for neutrophil elastase [6,17]. The local balance between proteinases and endogenous inhibitors like AAT is an important factor in determining whether inflammation results in connective tissue damage.

AAT is synthesised primarily in the liver, but also in extrahepatic tissues and cells, including neutrophils, monocytes and macrophages, alveolar macrophages, intestinal epithelial cells, carcinoma cells and the cornea [45–48]. AAT is found in most tissues and body fluids and it is an acute-phase reactant whose plasma concentration can rise by 3–4-fold above normal (1.34 mg/ml) during inflammation, infection and malignant diseases. In the human cornea, its local concentration is regulated by growth factors and might be increased up to 11-fold [48]. Although the mechanisms responsible for the increase of AAT are poorly understood, it has been shown that human neutrophils, monocytes and alveolar macrophages can increase expression of AAT in response to inflammatory mediators, such as interleukin-6, bacterial lipopolysaccharide and in response to AAT itself when complexed with neutrophil elastase [49,50].

Many data support the widely accepted notion that a major function of AAT is the inhibition of overexpressed proteinases during inflammatory processes [51–53]. However, it is also known that the biological activity of AAT can be affected by chemical modifications, including inter- or intramolecular polymerisation, oxidation, complex formation and cleavage by non-specific proteinases (Fig. 2).

3.1. Polymerisation

Molecular mobility confers on serpins the capacity to bind and entrap their target proteinases in highly stable complexes, but also a concomitant propensity to form dysfunctional molecules. A single amino acid

change in certain domains of the serpin molecule can block changes in structure necessary for normal inhibitory activity and folding and can lead to polymerisation of mutant serpin into intracellular aggregates [18,54]. A well-characterised example of a mutant serpin associated with a disease state is AAT, which occurs at low levels in early onset pulmonary emphysema [2]. The most widely studied deficiency variants of AAT are Z and S, which have E342K and E264V mutations respectively [54,55]. Polymerisation of AAT is also known to be involved in AAT deficiency-related diseases such as liver cirrhosis, neonatal hepatitis, hepatocellular carcinoma and lung emphysema [56–58]. These pathologies are characterised by the formation of intracellular inclusions of polymerised AAT due to sequential insertion of the reactive loop from one AAT molecule into a large β-sheet of another [59]. Although lung injury is believed to occur due to the decrease in elastase inhibitory capacity normally provided by AAT, liver injury is also thought to be due to the hepatotoxic effect of the abnormally folded AAT molecules retained in the endoplasmic reticulum (ER) [60]. The AAT Z variant has been demonstrated to polymerise spontaneously at higher concentrations and increased temperatures in vitro[61], suggesting that an increase in body temperature during systemic inflammation would exacerbate this tendency in vivo.

Recently a familial encephalopathy was described in which a mutant neuroserpin forms inclusion bodies [62]. Interestingly, the histochemistry of the neuroserpin aggregates, or Collins bodies, observed in the brain were found to be very similar to the AAT inclusions produced in the liver of those individuals expressing the Z variant [63]. Polymerisation also occurs in variants of other members of the serine proteinase inhibitor superfamily, such as antithrombin, C1-inhibitor, and α_1-antichymotrypsin, in association with thrombosis [64], angioedema [65], and chronic obstructive pulmonary disease [66], respectively. Cleavage of the reactive site loop at non-target positions also promotes polymerisation of AAT [67]. This polymerisation process may play a role in amyloidosis, since fragments of AAT have been shown to form β-sheet rich fibrils in vitro [68]. There are at least 15 human diseases associated with accumulation of specific proteins in insoluble polymers known as amyloid. Understanding the mechanism and structural determinants of protein polymerisation may open pathways to rational design of drugs to block the protein-protein associations and thereby ameliorate the associated disease. Recently, a class of compounds called chemical chaperones, such as 4-phenylbutyric acid, has been shown to reverse the cellular mislocalisation or misfolding of intracellular proteins and to mediate increased secretion of mutant AAT Z [69]. This might provide a pharmacological strategy for prevention of liver injury and emphysema in AAT deficiency.

Beyond the attendant loss of proteinase inhibitor activity, it is also possible that polymerised forms of serpins have other functional activities. Polymerised forms of various serpins have been shown to be present in human biological fluids from various inflammatory diseases, but their biological roles and elimination rates, and their effects on cellular functional activities have not been defined. Elucidation of the biological activities of polymeric forms of serpins may provide not only useful insights into the pathogenesis of amyloid-related and other diseases, but may also suggest therapeutic strategies.

3.2. Complex formation

AAT, like most inhibitory serpins, inhibits proteinases through the formation of a serpin-enzyme complex (Fig. 2). AAT contains a single reactive site, centred at a Met-Ser sequence 36 amino acid residues from the C-terminus. Formation of a 1:1 molar complex between serpin and enzyme is accompanied by cleavage in the reactive site of the AAT and an irreversible conformational transition to a stable, inactive form. When complexes are formed between the serpin and its target protease, there is an accompanying conformational change in the serpin that either unmasks or causes the formation of a new binding site in the complexed serpin that is not present in the free serpin [49,70]. Studies by many groups have shown that under normal physiological conditions, the liver mediates rapid clearance from the circulation of serpin-proteinase complexes, including AAT-proteinase complexes, via several receptors, such as the serpin-enzyme complex (SEC) receptors [71], low-density lipoprotein (LDL) receptor [72] and very-low-density lipoprotein (VLDL) receptor [73]. However, high levels of circulating AAT-elastase com-

plexes have been detected in patients with lung emphysema and rheumatic diseases [74] during acute processes such as myocardial infarction and acute leukaemia [75,76], and elevated levels of AAT-trypsin complexes have been found in patients with biliary tract cancer [77]. Thus, clearance of AAT-proteinase complexes appears to be impaired or inadequate in capacity in some pathological conditions, raising the possibility that these circulating serpin-proteinase complexes have biological functions. It was shown that these complexes stimulate the biosynthesis of AAT in cell cultures and are chemotactic for neutrophils [78]. Moreover, the observation that serpin-proteinase complexes are recognised by endocytosis receptors of the LDL receptor family suggests that those complexes may play a role in both lipid metabolism and proteinase activity regulation during the inflammatory response.

AAT is known to form complexes not only with target proteinases, but also with other molecules. Complexes between monoclonal immunoglobulin light chains of κ type and AAT have been found in serum from patients with myeloma and Bence-Jones proteinemia [79], and factor XIa-AAT and AAT-glucose complexes are common in plasma from diabetic subjects [80]. Disulphide linked complexes between immunoglobulin A (IgA) and AAT are detected at low levels in the sera of healthy volunteers, but are found at increased levels in the sera and synovial fluid of patients with rheumatoid arthritis (RA), systemic lupus erythematosus and ankylosing spondylitis [81]. Recently it has been suggested that alteration in AAT structure might be an important factor in promoting IgA-AAT complex formation, since AAT damaged by heating and low pH as occurs in normal urine, is able to complex more rapidly with IgA [82]. However, the reason for increased IgA-AAT formation in the circulation of patients with RA is still unknown. Prostate-specific antigen (PSA), itself a kallikrein-ACT complex, which correlates with prostate hypertrophy and malignancy, is also known to be bound to AAT in sera of subjects with high PSA concentrations [83]. These findings suggest that further unexpected causal links may exist between serpin complex formation with specific ligands, alterations in functional activity, and the pathological processes characterised by such complex formation.

3.3. Cleavage

Cleaved AAT is another non-inhibitory form found in vivo, whose other biological roles have received little attention (Fig. 2). Cleaved forms of AAT are know to occur when AAT forms an inhibitor complex with serine proteinase which subsequently dissociates or is degraded, or when it is cleaved by non-target proteinases, usually at sites in its reactive site, without formation of stable inhibitor complexes. Such cleavages generate a 4 kDa carboxyl-terminal fragment of 36 residues, which remains non-covalently bound to the cleaved AAT. Human cathepsin L, collagenase and stromelysin, and bacterial proteinases from *Staphylococcus aureus*, *Serratia marcescens* metalloproteinase and *Pseudomonas aeruginosa* elastase [84] all fall into the latter class and exhibit efficient AAT degrading activity. Recent studies established AAT as a key substrate for gelatinase B (MMP-9) in vivo [85]. It has long been hypothesised that neutrophil elastase-mediated tissue destruction in certain inflammatory diseases such as emphysema, is caused by an imbalance in the ratio of elastase to AAT [86]. The studies of Liu and collaborators provide in vivo evidence that this mechanism, mediated by the proteolytic inactivation of AAT by gelatinase B, underlies the pathology of an inflammatory skin disorder called bullous pemphigoid, that is initiated by deposit formation at the basement membrane [85,87]. Generated cleaved forms of AAT may contribute to the later phase of polymorphonuclear leucocyte infiltration. Indeed, cleaved AAT was shown to be a potent chemoattractant for monocytes [88]. Recently, the structure of a cleaved polymer of AAT was found to confirm the first proposed model of serpin polymerisation [89–91]. Moreover, two distinct β-sheet interactions (A- and C-sheet) of the cleaved AAT were observed in the polymers, and it was suggested that the A β-sheet interaction is likely to be important in serpin polymer formation, while the C β-sheet interaction may provide a possible explanation for serpin interaction with other proteins. Polymers of AAT and other serpins have been observed in vivo. Lomas and co-workers showed that Z AAT polymerised in vitro has identical properties and ultrastructure to the inclusions isolated from hepatocytes of a Z homozygote [92], but there are no other available data that identify intact or cleaved

serpin is involved in formation of various polymers. Therefore further studies are needed to establish which form of serpin is involved in polymer formation under various pathophysiological conditions.

3.4. Degradation

Cleaved, carboxyl-terminal fragments of AAT arising from non-target proteolytic cleavage were found to be present in human bile and in a variety of human tissues, such as placenta, pancreas, stomach and small intestine [46,93–95]. Increased levels of fragmented AAT in oral tissue and fluids were found in subjects with insulin-dependent diabetes mellitus (IDDM) who had been clinically diagnosed as having periodontal disease [96]. Also, the carboxyl-terminal fragment of AAT created by reactive site cleavage was found to be associated with extracellular matrix proteins such as collagen and/or laminin-1, and it was suggested that this AAT peptide plays an important role in the protection of these proteins from inappropriate enzyme digestion [97]. We know from various studies that the carboxyl-terminal segment remains firmly attached to the core of cleaved AAT and to the cleaved AAT in complex with enzyme [70,88]. Although the peptide is non-covalently bound to the remaining molecule of AAT, it can only be separated under denaturing condition [67,94,98]. This raises the question of how degradation fragments of serpins, like the carboxyl-terminal fragment of cleaved AAT, can be generated in free form. In vitro studies have shown that serpin-proteinase complexes are unstable, their rate of breakdown apparently dependent on their structure and environmental conditions. This increased susceptibility of serpin-proteinase complex to proteolytic degradation could be one possible source of the AAT fragments. Also, native AAT and, in particular, oxidised AAT, are known to be good substrates for metalloproteinases [84,85]. The cleavages of AAT by these proteinases might generate fragments of AAT. Endocytosis of serpins and serpin-proteinase complexes could also give rise to their degradation, with exocytosis of peptide fragments. Although these speculations remain to be proven, the fact that the peptide(s) of AAT can be detected in vivo confirms their dissociation from the parent serpin.

We have examined the effects of proteolytically cleaved AAT and the amyloidogenic carboxy-terminal fragment of AAT on hepatoma cells and monocyte cultures [99,100], and showed that these forms of AAT exert significant effects on cellular lipid catabolism and proinflammatory activation. These forms of AAT were also found to activate peroxisome proliferator-activated receptors (PPARs), transcription factors that recently have been proposed to regulate genes for lipid metabolism and proinflammatory proteins [100]. Furthermore, we determined that the carboxy-terminal cleavage fragment of AAT is a component of atherosclerotic plaque, located specifically in the fibrous cap near the necrotic core [100]. The proteolytic inhibitors AAT and α_2-macroglobulin have been shown to be present in relatively high concentrations in human aortic atherosclerotic lesions, and it has been suggested that these proteins could enhance fibrosis of the lesion because of their known inhibitory effects on elastase and collagenase [101,102]. Consistent with this, plasma levels of AAT are correlated with the development of both early and advanced atherosclerosis in the carotid arteries [103].

The mechanism by which AAT contributes to progressive coronary atherosclerosis is unknown, but it appears to accompany tissue injury and vascular inflammation, conditions which are mediated by a complex network of molecular interactions and increased expression of acute-phase proteins, including AAT. It is likely that proteinase inhibitory activity of AAT can be compromised by chemical modifications attendant on the inflammatory state, and the modified form(s) of AAT may also play a role in atherogenesis. The observed biological activities of the cleaved forms of AAT suggest that the role of AAT in the pathology of atherosclerosis is not only as an inhibitor of proteases, but also as a protease substrate and a reservoir of physiologically active peptide degradation products. Since atherosclerosis appears to be a chronic inflammatory process in the vessel wall in response to injury, the markedly increased biosynthesis of AAT during inflammation might give rise to a large fraction of cleaved/degraded forms of AAT. A proteinase-induced imbalance in favour of the latter could play a critical role in lesion progression.

Production of AAT by tumour cells has been reported [47,104], and earlier observations that AAT-

positive adenocarcinomas of colon and lung have a worse prognosis than AAT-negative ones [105] suggest that AAT may also play a role in cancer. Malignant cells are characterised by their ability to spread and invade normal tissues. Both of these processes involve proteolytic activities of malignant cells which detach the cell and degrade extracellular matrices. The proteases involved in these processes which have been characterised so far, include metalloproteinases (MMPs) produced by both the cancer cells and the surrounding host tissue. Since AAT is produced by various tumour cells and is a good substrate for MMPs [84,85], one can speculate that there is a link between MMP activity, tumour cell propensity to produce and secrete AAT, and tumour growth. Recent studies provide good experimental evidence that the C-terminal fragment of AAT generated by matrix metalloproteinases enhances tumour growth and invasiveness in vivo [106].

Together, these observations support the notion that under inflammatory conditions AAT may play a role not only as an inhibitor of proteases, but also as a protease substrate and a reservoir of physiologically active peptide degradation products. Studies also suggest that non-inhibitory forms of AAT, particularly the cleaved and/or degraded forms, may have yet undefined biological activities in inflammatory processes, such as atherosclerosis and cancerogenesis. Demonstration that serpins, including native and cleaved AAT molecules, form polymers, leads to the speculation that this polymerisation might conduce to both generation of biologically active peptides and to amyloid formation in amyloid-related diseases in which serpins are known to be involved.

3.5. Oxidation

Oxidation of methionine (Met) has been shown to affect a number of biologically active proteins [107]. Met oxidation may lead to an increase in biological activity, in case of the C5 component of complement [108], or to no apparent change in structure or activity in case of α_2-plasmin inhibitor [109]. It has also been shown that oxidation of the two most susceptible Met in another serpin, antithrombin, does not inactivate the protein nor affect its ability to bind heparin [107]. Oxidised AAT is a modified form of AAT often found in inflammatory exudates at levels of about 5–10% that of total AAT [110]. The amino acid at position P1 in the reactive site of each inhibitory serpin primarily determines the specificity of inhibition and thereby its biological activity. P1 in AAT is Met, the most readily oxidised amino acid of proteins, which is converted by oxidation to methionine sulphoxide [111]. Met can be attacked by various oxidants produced in biological systems, such as peroxide, hydroxyl radicals, hypochloride, chloramines and peroxynitrite [112]. It has been shown that levels of active circulating AAT can be reduced in normal, healthy individuals by enzymatic oxidation of the reactive site methionine of AAT by neutrophil myeloperoxidase. Also, oxidative inactivation of the AAT can be induced in vitro by incubating AAT with purified myeloperoxidase or stimulated phagocytes [113]. Evidence that this occurs in vivo comes from the observation that inactive AAT purified from inflammatory synovial fluid contains methionine sulphoxide residues [114]. Some bacteria can also exploit the oxidation of AAT. This is apparent for *S. marcescens* and *S. aureus* metalloproteinases, which cleave oxidised AAT significantly faster than native [115]. Thus, oxidation and proteolytic processes in some cases may work synergistically to promote local inflammation, including uncontrolled degradation of connective tissues. That AAT oxidation and proteolysis do occur in vivo is supported by findings that, on average, 41% of total AAT in rheumatoid arthritis synovial fluid is inactive, oxidised and/or cleaved [116]. Recently it has been shown that oxidation of AAT promotes AAT-immunoglobulin A complex formation in vitro. IgA-oxidised AAT complexes isolated from synovial fluid of rheumatoid disease patients were suggested to protect the oxidised AAT molecule from proteolytic cleavage by free elastase [81,117], and to be important in maintaining the balance of proteinase and antiproteinase levels during inflammation, especially if dissociation of IgA-oxidised AAT complex can occur under conditions that lead to reactivation of oxidised AAT.

Oxidative inactivation of AAT with subsequent enhanced proteolysis, particularly by neutrophil elastase, has been invoked in the pathogenesis of pulmonary emphysema in smokers [118–120]. AAT recovered in bronchial lavage fluid from human and rat lungs after exposure to cigarette smoke shows it to

contain methionine sulphoxide residues and to have diminished inhibitory capacity. Similarly, lavage fluid from the lungs of smokers showed 2-fold diminution in AAT activity relative to that from non-smokers [119,121,122].

Leucocytes, neutrophils and macrophages, which secrete large quantities of oxidants at sites of inflammation, were shown to induce oxidative inactivation of AAT in vivo and to result in perturbed protease-antiprotease balance. Although oxidised AAT plays a proinflammatory role at sites of inflammation because of its loss of inhibitor activity toward proteases, it cannot be excluded that oxidised AAT may also have other biological activities related to inflammation. Using primary human monocytes cultures, we have found that oxidised AAT induces monocyte activation and alterations in cholesterol homeostasis [123], which provides evidence that generation of oxidised AAT in inflammatory loci may play a role in modulating inflammatory responses.

4. Serpins in neurodegenerative brain diseases

Interest in serine and cysteine proteinases and their serpin inhibitors, and their roles in the pathogenesis of neurodegenerative diseases has expanded recently, because these proteinases and their inhibitors were detected at sites of neuronal injury. Numerous studies have been conducted to determine the role of serine proteases and serpins in physiologic and pathologic conditions. Serine proteinases have been detected in neurones and in glial cells of the central nervous system (CNS) and shown to regulate the balance of accumulation and degradation of the extracellular matrix. For example, the serine proteinase thrombin was shown to protect astrocytes against hypoglycaemia or oxidative stress, but to increase neuronal death, while tissue-type plasminogen activator (t-PA) was shown to protect neurones against Zn-induced cell death, but to mediate neuronal death induced by excitotoxins [124–126].

It is inferred from different studies that expression of serine proteinases is modulated during brain injury and that an impairment in the balance between serine proteinases and their cognate inhibitors in the CNS may lead to a pathologic state [125,127]. Therefore, the levels of expression of native and chemically modified forms of serpins may be important in understanding various neurodegenerative conditions. It has been reported that the expression of the type 1 PAI and protease nexin-1 (PN-1) is increased in the cerebrospinal fluid from patients with neurologic disorders, such as Alzheimer's disease (AD), cerebral infarction, CNS infection and neoplasia [125]. Increased levels of other members of the serpin family, such as antithrombin, an inhibitor of the blood coagulation cascade, and ACT, an inhibitor of proteinases of the chymotrypsin class, were also found in AD plaques [128,129]. Several observations have suggested that serpin levels might influence the outcome of brain injury. For example PAI-1 produced by astrocytes was found to be neuroprotective against necrosis mediated *N*-methyl-D-aspartate [130]. PN-1 was reported to protect cultured neurones from glucose deprivation-induced damage through attenuation of the increase in intracellular calcium levels associated with such damage [131].

Neuroserpin is a newly identified member of the serpin family that is primarily expressed in brain, and primarily localised to neurones [132]. Neuroserpin is believed to play a vital role in controlling extracellular proteolysis in the nervous system, especially as an inhibitor of tissue-type plasminogen activator [133]. As such, neuroserpin is also suggested to be an important factor contributing to neuronal plasticity and learning [134]. Recently it was shown that mutations in the neuroserpin gene result in polymerisation and aggregation of the neuroserpin protein, with consequent diminution in neuroserpin levels, which might result in uncontrolled proteolysis and the loss of neuronal function [135,136]. On the other hand, it is equally conceivable that the inclusion bodies formed from the aggregated neuroserpin, so-called Collins bodies [62], themselves are neurotoxic. The newly described familial encephalopathy with neuroserpin inclusion bodies provides additional suggestive evidence that the inclusion bodies themselves have pathological effects.

5. α_1-Antichymotrypsin: link to Alzheimer's disease

A link between ACT and AD was established by the observation that ACT is present in both amorphous and classic plaques of AD and in amyloid

deposits in Down's syndrome and normally aged brains [137,138]. ACT is an acute phase protein and a serpin proteinase inhibitor, which is synthesised primarily by hepatocytes, bronchial epithelial cells, activated astrocytes and to a small extent by monocytes [139–141]. ACT appears to specifically inactivate neutrophil cathepsin G, mast cell chymase and pancreatic chymotrypsin [142]. In addition, it has been shown to reduce the natural killing activity of T-cell lymphocytes and to inhibit superoxide generation by human neutrophils [143]. The major fraction of immunologically identifiable human prostate derived proteases, used clinically to monitor patients with prostate cancer, is found in complex with ACT [144].

To determine the state in which ACT is deposited in amyloid plaques, monoclonal antibodies against ACT that can distinguish native ACT from complexed and inactive were used, and it was found that ACT in both amorphous and classic plaques is either complexed or proteolytically inactive [145]. ACT, like other serpins, is characterised by a mobile reactive site and a dominant β-sheet A which can open to insert the cleaved active site loop (Fig. 1). The intact serpins can also take up exogenous free peptides of the same or similar sequence as their reactive site sequence into β-sheet A upon incubation with excess peptide [146]. Such an interaction results in inactivation and polymerisation of serpin. AD amyloid plaques are composed primarily of variants of the β-amyloid protein (Aβ), which is a proteolytic fragment of the larger β-amyloid precursor protein [147]. We and others have shown that ACT forms extremely stable complexes with $A\beta_{1-42}$, comparable in specificity and stability to a proteinase-inhibitor interaction, and that it also inhibits or accelerates fibrillisation of $A\beta_{1-42}$ [148–150]. Furthermore, the binding of Aβ to ACT renders ACT inactive as a proteinase inhibitor. Thus, ACT acts as a molecular chaperone to influence fibril formation through specific complexes with $A\beta_{1-42}$, and $A\beta_{1-42}$ reciprocally abolishes ACT inhibitor activity. The loss of ACT inhibitor activity implies dysregulation of proteinase(s) at foci of Aβ biosynthesis, these proteinases being elevated during inflammation associated with AD.

ACT-chymotrypsin complexes regulate neutrophil superoxide production [143] and AAT-proteinase complex has neutrophil chemoattractant activity and upregulates synthesis of AAT [78]. The complex of $A\beta_{1-42}$ with ACT structurally resembles that of serpin-proteinase complexes, and since the latter have biological activities beyond that of proteinase inhibition, it is possible that ACT-$A\beta_{1-42}$ complexes also have as yet undiscovered biological activities which contribute to the establishment of self-propagating neurotoxic pathologies. ACT-proteinase complexes and/or ACT-Aβ complex may feedback upregulate ACT biosynthesis, which by raising local ACT levels in the presence of increasing Aβ could sustain pathological cycles [151]. Proteinase targets of ACT which are unregulated as a result of Aβ blockage of ACT inhibitor activity could include proteinases associated with $A\beta_{1-42}$ biosynthesis or degradation, and also proteinases linked to cytokine activity. It was recently shown that ACT can indirectly inhibit a proteinase which degrades $A\beta_{1-42}$ [148].

Serine proteinases and their inhibitors are extensively involved in inflammatory processes related to neurodegenerative diseases. Little is known about the metabolic fate and biological activities of ACT-proteinase complexes and nothing about non-inhibitory ACT-Aβ complexes, but the notion that these complexes play roles in neurodegenerative disease merits further attention.

6. Conclusion

Virtually all proteins function by interacting with other molecules and these interactions may alter the physical, biochemical and functional properties of the proteins. The capacity to undergo conformational change is crucial for the physiological function of many proteins, and the serpins are a clear case where such changes have been exploited during evolution as a means of modulating inhibitory activity. This versatility of structure and function may also explain how in complex environments the serpins have become the predominant proteinase inhibitors in higher organisms. The chemical reactivity, molecular structure and physical properties of serpins permit these proteins to adopt a number of variant conformations and assembly states under physiological conditions. Inflammatory exudates contain serine proteinase inhibitors in diverse molecular forms, including a na-

tive inhibitory form and several inactive, non-inhibitory forms, such as complexes with protease or other ligands, cleaved, polymerised and oxidised. Although these forms of inhibitory serpins are detected in tissues and fluids recovered from inflammatory sites, the important questions of which conditions result in generation of different molecular forms of serpins, what biological function these forms have, and which of them are directly linked to pathologies and/or may be useful markers for characterisation of disease state, remain to be answered. On the other hand, an appreciation that one and the same serpin is able to perform multiple functions dependent on its molecular form will be necessary to achieve an understanding of new important roles of serpins in the coming years.

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References

[1] R.A. Engh, A.J. Schulze, R. Huber, W. Bode, Behring. Inst. Mitt. 93 (1993) 41–62.
[2] C.B. Laurell, S. Eriksson, Scand. J. Clin. Lab. Invest. 15 (1963) 132–140.
[3] J.J. Bao, R.N. Sifers, V.J. Kidd, F.D. Ledley, S.L. Woo, Biochemistry 26 (1987) 7755–7759.
[4] L.T. Hunt, M.O. Dayhoff, Biochem. Biophys. Res. Commun. 95 (1980) 864–871.
[5] R.W. Carrell, P.A. Pemberton, D.R. Boswell, Cold Spring Harbor Symp. Quant. Biol. 52 (1987) 527–535.
[6] R. Carrell, J. Travis, Trends Biochem. Sci. 10 (1985) 20–24.
[7] J. Potempa, E. Korzus, J. Travis, J. Biol. Chem. 269 (1994) 15957–15960.
[8] G.L. Hammond, Endocr. Rev. 11 (1990) 65–79.
[9] G.L. Hammond, C.L. Smith, I.S. Goping, D.A. Underhill, M.J. Harley, J. Reventos, N.A. Musto, G.L. Gunsalus, C.W. Bardin, Proc. Natl. Acad. Sci. USA 84 (1987) 5153–5157.
[10] P.E. Stein, D.A. Tewkesbury, R.W. Carrell, Biochem. J. 262 (1989) 103–107.
[11] P.I. Bird, Results Probl. Cell Differ. 24 (1998) 63–89.
[12] D.M. Worrall, O.E. Blacque, R.C. Barnes, Biochem. Soc. Trans. 4 (1999) 746–750.
[13] Z. Zou, A. Anisowicz, M.J. Hendrix, A. Thor, M. Neveu, S. Sheng, K. Rafidi, E. Seftor, R. Sager, Science 263 (1994) 526–529.
[14] M. Zhang, O. Volpert, Y.H. Shi, N. Bouck, Nat. Med. 6 (2000) 196–199.
[15] M. Riewald, R.R. Schleef, J. Biol. Chem. 270 (1995) 26754–26757.
[16] J. Sun, J.B. Rose, P.J. Bird, Biol. Chem. 270 (1995) 16089–16096.
[17] J.L. Macen, C. Upton, N. Nation, G. McFadden, Virology 195 (1993) 348–363.
[18] P. Nash, A. Whitty, J. Handwerker, J. Macen, G. McFadden, J. Biol. Chem. 273 (1998) 20982–20991.
[19] J. Travis, G.S. Salvesen, Annu. Rev. Biochem. 52 (1983) 655–709.
[20] P.E. Stein, R.W. Carrell, Struct. Biol. 2 (1995) 96–113.
[21] H. Lobermann, R. Tokuoka, J. Deisenhofer, R. Huber, J. Mol. Biol. 177 (1984) 531–557.
[22] J.A. Huntington, R.J. Read, R.W. Carrell, Nature 407 (2000) 923–926.
[23] M. Herve, C. Ghelis, Arch. Biochem. Biophys. 285 (1991) 142–146.
[24] E.S. Stavridi, K. O'Malley, C.M. Lukacs, W.T. Moore, J.D. Lambris, D.W. Christianson, H. Rubin, B.S. Cooperman, Biochemistry 35 (1996) 10608–10615.
[25] G. Kaslik, J. Kardos, E. Szabo, L. Szilagyi, P. Zavodszky, W.M. Westler, J.L. Markley, L. Graf, Biochemistry 36 (1997) 5455–5464.
[26] H.T. Wright, J.N. Scarsdale, Proteins Struct. Funct. Genet. 22 (1995) 210–225.
[27] P.C. Hopkins, S.R. Stone, Biochemistry 34 (1995) 15872–15879.
[28] D.A. Lane, R.R. Olds, S.L. Thein, Blood Coagul. Fibrinolysis 3 (1992) 315–341.
[29] D.H. Perlmutter, Ann. Med. 28 (1996) 385–394.
[30] M. Yazaki, J.J. Liepnieks, J.R. Murrell, M. Takao, B. Guenther, P. Piccardo, M.R. Farlow, B. Ghetti, M.D. Benson, Am. J. Pathol. 58 (2001) 227–233.
[31] E.R. Jordan, J. Kilpatrick, R.M. Nelson, Science 237 (1987) 777–779.
[32] B. Wiman, A. Almquist, O. Sigurdardottir, T. Lindahl, FEBS Lett. 242 (1988) 125–128.
[33] S. Stefansson, D.A. Lawrence, Nature 383 (1996) 441–443.
[34] Y. Miyamoto, T. Akaike, M.S. Alam, K. Inoue, T. Hamamoto, N. Ikebe, J. Yoshitake, T. Okamoto, H. Maeda, Biochem. Biophys. Res. Commun. 267 (2000) 918–923.
[35] S. Takada, M. Tsuda, M. Yamamura, T. Katsunuma, Biochem. Int. 16 (1988) 949–954.
[36] M. Tsuda, M. Masuyama, T. Katsunuma, Cancer Res. 46 (1986) 6139–6142.
[37] S. Qing-Bai, J.J. Mukherjee, K.S. Crilly, Z. Kiss, FEBS Lett. 473 (2000) 33–36.
[38] J. Yao, C.M. Baecher-Allan, J. Sharon, Mol. Cell Biol. Res. Commun. 3 (2000) 76–81.
[39] J.J. Enghild, Z. Valnickova, I.B. Thogersen, S.V. Pizzo, J. Biol. Chem. 269 (1994) 20159–20166.
[40] T.W. Stief, A. Aab, N. Heimburger, Thromb. Res. 49 (1988) 581–589.

[41] D.C. Crowther, D.L. Evans, R.W. Carrell, Curr. Opin. Biotechnol. 3 (1992) 399–407.
[42] M.S. O'Reilly, S. Pirie-Shepherd, W.S. Lane, J. Folkman, Science 285 (1999) 1926–1928.
[43] H.F. Dvorak, New Engl. J. Med. 315 (1986) 1650–1659.
[44] J.R. Westphal, R. Van't Hullenaar, A. Geurts-Moespot, F.C. Sweep, J.H. Verheijen, M.M. Bussemakers, J. Askaa, I. Clemmensen, A.A. Eggermont, D.J. Ruiter, R.M. De Waal, Int. J. Cancer 86 (2000) 760–767.
[45] M.B. Ray, K. Geboes, F. Callea, V.J. Desmer, Cell Tissue Res. 185 (1977) 63–68.
[46] K. Geboes, M.B. Ray, P. Rutgeerts, F. Callea, V.J. Desmet, G. Vantrappen, Histopathology 6 (1982) 55–60.
[47] D. Keppler, M. Markert, B. Carnal, J. Berdoz, J. Bamat, B. Sordat, Biol. Chem. Hoppe Seyler 377 (1996) 301–311.
[48] G. Boskovic, S.S. Twining, Biochim. Biophys. Acta 1403 (1998) 37–46.
[49] D.H. Perlmutter, P.I. Punsal, J. Biol. Chem. 263 (1988) 16499–16503.
[50] D.L. Knoell, D.R. Ralston, K.R. Coulter, M.D. Wewers, Am. J. Respir. Crit. Care Med. 157 (1998) 246–255.
[51] J. Travis, Am. J. Med. 84 (1988) 37–42.
[52] D.A. Lomas, R.W. Carrell, Am. J. Physiol. 265 (1993) L211–L219.
[53] N.A. Kalsheker, QJM Mon. J. Assoc. Phys. 87 (1994) 653–658.
[54] R.W. Carrell, D.A. Lomas, S. Sidhar, R. Foreman, Chest 110 (1996) 243S–247S.
[55] R.W. Carrell, J.O. Jeppsson, C.B. Laurell, S.O. Brennan, M.C. Owen, L. Vaughan, D.R. Boswell, Nature 298 (1982) 329–334.
[56] S. Eriksson, Lung 168 (1990) 523–529.
[57] T. Sveger, New Engl. J. Med. 294 (1976) 1316–1321.
[58] R. Durr, W.H. Caselmann, Langenbeck's Arch. Surg. 385 (2000) 154–161.
[59] T.R. Dafforn, R. Mahadeva, P.R. Elliott, P. Sivasothy, D.A. Lomas, J. Biol. Chem. 274 (1999) 9548–9555.
[60] D. H Perlmutter, F.S. Cole, T.H. Kilbridge, T.H. Rossing, H. R Colten, Proc. Natl. Acad. Sci. USA 82 (1985) 795–799.
[61] D.A. Lomas, D.L. Evans, S.R. Stone, W.S. Chang, R.W. Carrell, Biochemistry 32 (1993) 500–508.
[62] R.L. Davis, P.D. Holohan, A.E. Shrimpton, A.H. Tatum, J. Daucher, G.H. Collins, R. Todd, C. Bradshaw, P. Kent, D. Feiglin, A. Rosenbaum, M.S. Yerby, C.M. Shaw, F. Lacbawan, D.A. Lawrence, Am. J. Pathol. 155 (1999) 1901–1913.
[63] R.L. Davis, A.E. Shrimpton, P.D. Holohan, C. Bradshaw, D. Feiglin, G.H. Collins, P. Sonderegger, J. Kinter, L.M. Becker, F. Lacbawan, D. Krasnewich, M. Muenke, D.A. Lawrence, M.S. Yerby, C.M. Shaw, B. Gooptu, P.R. Elliott, J.T. Finch, R.W. Carrell, D.A. Lomas, Nature 401 (1999) 376–379.
[64] M.W. Mosesson, Semin. Thromb. Hemost. 25 (1999) 311–319.
[65] H. Ono, H. Kawaguchi, N. Ishii, H. Nakajima, Hum. Genet. 98 (1996) 452–453.
[66] B. Gooptu, B. Hazes, W.S. Chang, T.R. Dafforn, R.W. Carrell, R.J. Read, D.A. Lomas, Proc. Natl. Acad. Sci. USA 97 (2000) 67–72.
[67] J.C. Whisstock, R. Skinner, R.W. Carrell, A.M. Lesk, J. Mol. Biol. 295 (2000) 651–665.
[68] S. Janciauskiene, E. Carlemalm, S. Eriksson, Biol. Chem. Hoppe Seyler 376 (1995) 415–423.
[69] J.A.J. Burrows, L.K. Willis, D.H. Perlmutter, Proc. Natl. Acad. Sci. USA 97 (2000) 1796–1801.
[70] E.W. Howard, D.J. Knauer, J. Cell. Physiol. 131 (1987) 276–283.
[71] D.H. Perlmutter, G.I. Glover, M. Rivetna, C.S. Schasteen, R.J. Fallon, Proc. Natl. Acad. Sci. USA 87 (1990) 3753–3757.
[72] M.Z. Kounnas, F.C. Church, W.S. Argraves, D.K. Strickland, J. Biol. Chem. 271 (1996) 6523–6529.
[73] K.W. Rodenburg, L. Kjoller, H.H. Petersen, P.A. Andeasen, Biochem. J. 329 (1998) 55–63.
[74] C. Beyeler, R.E. Banks, H.A. Bird, J. Rheumatol. 27 (2000) 15–19.
[75] E. Hiller, M. Jochum, Blut 48 (1984) 269–275.
[76] R. Egbring, R. Seitz, H. Blanke, J. Leititis, H.J. Kesper, R. Burghard, G. Fuchs, L. Lerch, Behring. Inst. Mitt. 79 (1986) 87–103.
[77] J. Hedstrom, C. Haglund, E. Kemppainen, M. Leinimaa, J. Leinonen, U.H. Stenman, Clin. Chem. 45 (1999) 1768–1773.
[78] G. Joslin, G.L. Griffin, A.M. August, S. Adams, R.J. Fallon, R.M. Senior, D.H. Perlmutter, J. Clin. Invest. 90 (1992) 1150–1154.
[79] C.B. Laurell, E. Thulin, Immunochemistry 11 (1974) 703–709.
[80] T. Murakami, Y. Komiyama, M. Masuda, M. Karakawa, T. Iwasaka, H. Takahashi, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 1107–1113.
[81] L.J. Scott, E.L. Evans, P.T. Dawes, G.I. Russell, D.L. Mattey, Br. J. Rheum. 37 (1998) 398–404.
[82] F.A. Wollheim, J.O. Jeppsson, C.B. Laurell, Scand. J. Rheumatol. 28 (Suppl.) (1979) 21–27.
[83] W. M Zhang, P. Finne, J. Leinonen, S. Vesalainen, S. Nordling, U.H. Stenman, Clin. Chem. 45 (1999) 814–821.
[84] M. Rapala-Kozik, J. Potempa, D. Nelson, A. Kozik, J. Travis, Biol. Chem. 380 (1999) 1211–1216.
[85] Z. Liu, X. Zhou, S.D. Shapiro, J.M. Shipley, S.S. Twining, L.A. Diaz, R.M. Senior, Z. Werb, Cell 102 (2000) 647–655.
[86] S.J. Weiss, New Engl. J. Med. 320 (1989) 365–376.
[87] R.E. Jordon, S. Kawana, K.A. Fritz, J. Invest. Dermatol. 85 (1985) 72s–78s.
[88] M.J. Banda, A.G. Rice, G.L. Griffin, R.M. Senior, J. Biol. Chem. 263 (1988) 4481–4484.
[89] M.A. Dunstone, W. Dai, J.C. Whisstock, J. Rossjohn, R.N. Pike, S.C. Feil, B.F. Le Bonniec, M.W. Parker, S.P. Bottomley, Protein Sci. 9 (2000) 417–420.
[90] J.A. Huntington, N.S. Pannu, B. Hazes, R.J. Read, D.A. Lomas, R.W. Carrell, J. Mol. Biol. 293 (1999) 449–455.
[91] A.E. Mast, J.J. Enghild, G. Salvesen, Biochemistry 31 (1992) 2720–2728.

[92] D.A. Lomas, D.L. Evans, J.T. Finch, R.W. Carrell, Nature 357 (1992) 605–607.
[93] M. Stark, H. Jornvall, J. Johansson, Eur. J. Biochem. 266 (1999) 209–214.
[94] J. Johansson, S. Gröndal, J. Sjövall, H. Jörnvall, T. Curstedt, FEBS Lett. 299 (1992) 146–148.
[95] M.A. Niemann, A.J. Narkates, A.J. Miller, Matrix 12 (1992) 233–241.
[96] C.L. Bristow, F. Di Meo, R.R. Arnold, Clin. Immunol. Immunopathol. 89 (1998) 247–259.
[97] M.A. Niemann, J.E. Baggott, E.J. Miller, J. Cell. Biochem. 66 (1997) 346–357.
[98] H. Im, H.Y. Ahn, M.H. Yu, Protein Sci. 9 (2000) 1497–1502.
[99] S. Janciauskiene, H.T. Wright, S. Lindgren, Atherosclerosis 147 (1999) 263–275.
[100] W. Dichtl, F. Moraga, M.P.S. Ares, M. Crisby, J. Nilsson, S. Lindgren, S. Janciauskiene, Mol. Cell Biol. Res. Commun. 4 (2000) 50–61.
[101] E.B. Smith, Am. J. Pathol. 86 (1977) 665–674.
[102] T. Mori, J. Sasaki, H. Kawaguchi, K. Handa, Y. Takada, A. Matsunaga, S. Kono, K. Arakawa, Am. Heart J. 129 (1995) 234–238.
[103] W. Hollander, M.A. Colombo, B. Kirkpatrick, J. Paddock, Atherosclerosis 34 (1979) 391–405.
[104] S. Tamir, S.S. Kadner, J. Katz, T.H. Finlay, Endocrinology 127 (1990) 1319–1328.
[105] S. Karashima, H. Kataoka, H. Itoh, R. Maruyama, M. Koono, Int. J. Cancer 45 (1990) 244–250.
[106] H. Kataoka, H. Uchino, T. Iwamura, M. Seiki, K. Nabeshima, M. Koono, Am. J. Pathol. 154 (1999) 457–468.
[107] S.M. Van Patten, E. Hanson, R. Bernasconi, K. Zhang, P. Manavalan, E.S. Cole, J.M. McPherson, T. Edmunds, J. Biol. Chem. 274 (1999) 10268–10276.
[108] W. Vogt, B. Zimmermann, D. Hesse, R. Nolte, Mol. Immunol. 29 (1992) 251–256.
[109] B.H. Shieh, J. Travis, J. Biol. Chem. 262 (1987) 6055–6059.
[110] P.S. Wong, J. Travis, Biochem. Biophys. Res. Commun. 96 (1980) 1449–1454.
[111] M.W. Swaim, S.V. Pizzo, J. Leukocyte Biol. 43 (1988) 365–379.
[112] W. Vogt, Free Radic. Biol. Med. 18 (1995) 93–105.
[113] A. Shock, H. Baum, Cell Biochem. Funct. 6 (1988) 13–23.
[114] Z. Zhang, A.J. Farrell, D.R. Blake, K. Chidwick, P.G. Winyard, FEBS Lett. 321 (1993) 274–278.
[115] Z. Zhang, P.G. Winyard, K. Chidwick, G. Murphy, M. Wardell, R.W. Carrell, D.R. Blake, Biochim. Biophys. Acta 1199 (1994) 224–228.
[116] K. Chidwick, P.G. Winyard, Z. Zhang, A.J. Farrell, D.R. Blake, Ann. Rheum. Dis. 50 (1991) 915–916.
[117] L.J. Scott, G.I. Russell, N.B. Nixon, P.T. Dawes, D.L. Mattey, Biochem. Biophys. Res. Commun. 255 (1999) 562–567.
[118] H.M. Morrison, Clin. Sci. (Colch) 2 (1987) 151–158.
[119] J.M. Sethi, C.L. Rochester, Clin. Chest Med. 21 (2000) 67–86.
[120] H.M. Morrison, D. Burnett, R.A. Stockley, Biol. Chem. Hoppe Seyler 367 (1986) 371–378.
[121] S.C. Afford, D. Burnett, E.J. Campbell, J.D. Cury, R.A. Stockley, Biol. Chem. Hoppe Seyler 369 (1988) 1065–1074.
[122] T. Matsuse, Y. Fukuchi, H. Matsui, E. Sudo, T. Nagase, H. Orimo, Chest 107 (1995) 395–400.
[123] F. Moraga, S. Janciauskiene, J. Biol. Chem. 275 (2000) 7693–7700.
[124] B.W. Festoff, I.V. Smirnova, J. Ma, B.A. Citron, Semin. Thromb. Hemost. 22 (1996) 267–271.
[125] D. Vivien, A. Buisson, J. Cereb. Blood Flow Metab. 20 (2000) 755–764.
[126] M.B. Gingrich, S.F. Traynelis, Trends Neurosci. 23 (2000) 399–407.
[127] M.L. Cuzner, G. Opdenakker, J. Neuroimmunol. 94 (1999) 1–14.
[128] R.N. Kalaria, T. Golde, S.N. Kroon, G. Perry, Am. J. Pathol. 143 (1993) 886–893.
[129] C.R. Abraham, T. Shirahama, H. Potter, Neurobiol. Aging 1 (1990) 123–129.
[130] A. Buisson, O. Nicole, F. Docagne, H. Sartelet, E.T. Mackenzie, D. Vivien, FASEB J. 12 (1998) 1683–1691.
[131] V.L. Smith-Swintosky, S. Zimmer, J.W. Fenton II, M.P. Mattson, J. Neurosci. 15 (1995) 5840–5850.
[132] T. Osterwalder, J. Contartese, E.T. Stoeckli, T.B. Kuhn, P. Sonderegger, EMBO J. 15 (1996) 2944–2953.
[133] T. Osterwalder, P. Cinelli, A. Baici, A. Pennella, S.R. Krueger, S.P. Schrimpf, M. Meins, P. Sonderegger, J. Biol. Chem. 273 (1998) 2312–2321.
[134] N.W. Seeds, B.L. Williams, P.C. Bickford, Science 270 (1995) 1992–1994.
[135] M. Yazaki, J.J. Liepnieks, J.R. Murrell, M. Takao, B. Guenther, P. Piccardo, M.R. Farlow, B. Ghetti, M.D. Benson, Am. J. Pathol. 158 (2001) 227–233.
[136] M. Takao, M.D. Benson, J.R. Murrell, M. Yazaki, P. Piccardo, F.W. Unverzagt, R.L. Davis, P.D. Holohan, D.A. Lawrence, R. Richardson, M.R. Farlow, B. Ghetti, J. Neuropathol. Exp. Neurol. 59 (2000) 1070–1086.
[137] C.R. Abraham, H. Potter, Ann. Med. 21 (1989) 77–81.
[138] C.R. Abraham, B.L. Razzaboni, S.S. Sisodia, E.H. Koo, D.L. Price, W.E. Van Nostrand, G. Papastoitsis, Ann. NY Acad. Sci. 640 (1991) 161–165.
[139] R.A. Stockley, Clin. Sci. (Colch) 64 (1983) 119–126.
[140] T. Kordula, M. Bugno, R.E. Rydel, J. Travis, J. Neurosci. 20 (2000) 7510–7516.
[141] D. Bergman, S.S. Kadner, M.R. Cruz, A.L. Esterman, M.M. Tahery, B.K. Young, T.H. Finlay, Pediatr. Res. 34 (1993) 312–317.
[142] N.A. Kalsheker, Int. J. Biochem. Cell Biol. 28 (1996) 961–964.
[143] L. Kilpatrick, J.L. Johnson, E.B. Nickbarg, Z.M. Wang, T.F. Clifford, M. Banach, B.S. Cooperman, S.D. Douglas, H. Rubin, J. Immunol. 146 (1991) 2388–23893.
[144] U.H. Stenman, J. Leinonen, W.M. Zhang, P. Finne, Semin. Cancer Biol. 9 (1999) 83–93.
[145] J.M. Rozemuller, J.J. Abbink, A.M. Kamp, F.C. Stam,

C.E. Hack, P. Eikelenboom, Acta Neuropathol. 82 (1991) 200–207.
[146] A.J. Schulze, P.W. Frohnert, R.A. Engh, R. Huber, Biochemistry 31 (1992) 7560–7565.
[147] S. Varadarajan, S. Yatin, M. Aksenova, D.A. Butterfield, J. Struct. Biol. 130 (2000) 184–208.
[148] R. Yamin, E.G. Malgeri, J.A. Sloane, W.T. McGraw, C.R. Abraham, J. Biol. Chem. 274 (1999) 18777–18784.
[149] J. McLaurin, D. Yang, C.M. Yip, P.E. Fraser, J. Struct. Biol. 130 (2000) 259–270.
[150] S. Eriksson, S. Janciauskiene, L. Lannfelt, Proc. Natl. Acad. Sci. USA 92 (1995) 2313–2317.
[151] S. Janciauskiene, H.T. Wright, BioEssays 20 (1998) 1039–1046.