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Review

Multi-functional capability of proteins: α1-antichymotrypsin and the correlation with Alzheimer's disease

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1. Introduction

An individual human is intrinsically capable of performing multitasks, sometimes, one at a time and other times, multiple at once. A talented musician can often play several different instruments. A composer and conductor can not only play several instruments, but he can also compose and conduct, for the diverse activities. This multitask phenomenon is not likely limited to human beings.

It has become increasingly clear that a large number of biological molecules can also perform multitasks. For example, RNA molecules can not only carry genetic information, but also catalyze a number of biochemical reactions [9,18]. Could this be true for proteins? The answer is likely to be yes. Proteins are sophisticated molecules with such a diversity of functions, from enzymes that catalyze reactions in a seemingly improbable environment at a rate of several orders of magnitude, we can only envy [16], to construct tough structural scaffolds on which all cells and living systems rely to survive. Recent observations of a variety of multitask capabilities of proteins provide us with an interesting glimpse on what will likely become increasingly important roles of proteins [2,51,62].

Virtually all proteins function by interacting with other molecules and these interactions can have numerous effects on the physical, structural, biochemical and functional properties of proteins. There are also different types of interactions between protein-proteins and protein-environment which likely lead to a complex formation, protein degradation, self-assembly or other modifications in protein structures. The ability to undergo post-translationally conformational changes is crucial for the physiological function of many proteins. On the other hand, such changes could alter both physicochemical and functional properties of the proteins with potential unforeseen physiological or pathological consequences.

2. Multi-functional capability of serpins

Among proteins the serine protease inhibitors (serpins) provide an excellent model for the structural and

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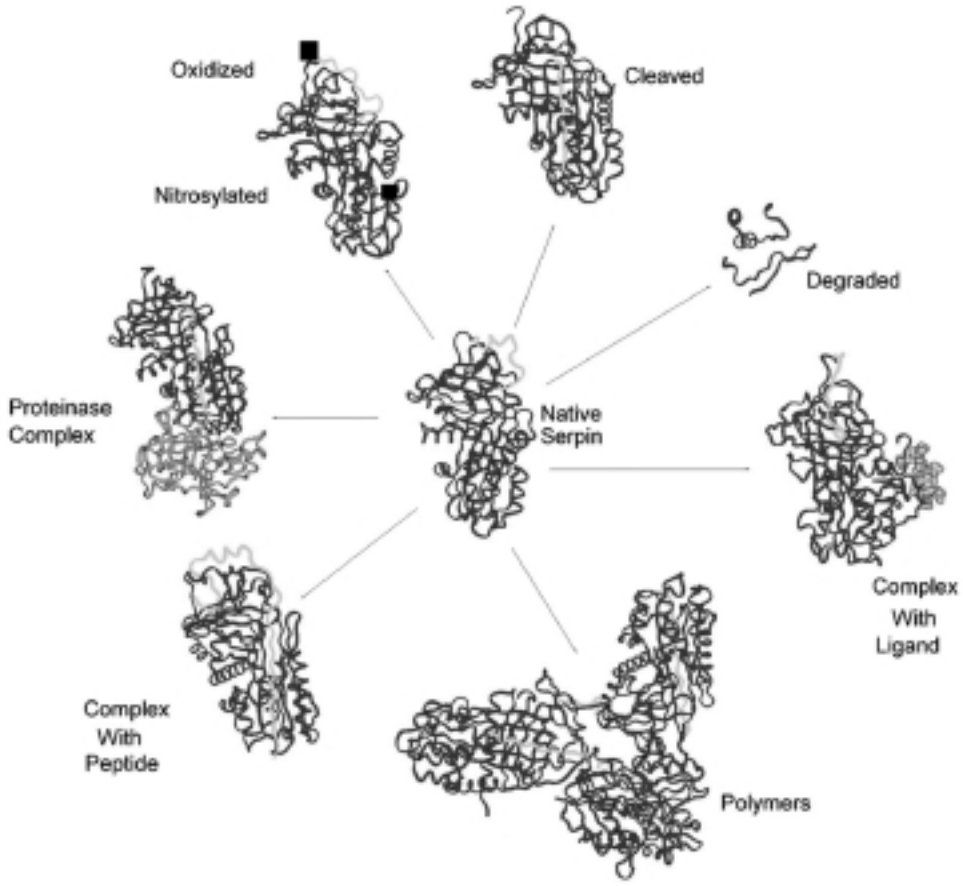

Fig. 1.

functional activity studies. They belong to a class of proteins that have shown to be capable of multitask performance. The serpins as natural inhibitors of serine proteases are well known and have been extensively studied [7,55]. They inhibit the over-expressed protease activity in order to protect tissue self-destruction. Serpins are characterized by the presence of a proteolytically sensitive reactive site loop that acts as an ideal substrate for a specific protease [55,60]. The molecular structure and physical properties of serpins allow them to adopt a number of variant conformations and assembly states under physiological conditions [7, 8]. Inhibitory serpins in biological fluids can exist in several forms: the native, inhibitory active, or non-inhibitory forms such as complexed with protease or other molecules, cleaved, degraded, oxidized and self-assembled [13,22,23] (Fig. 1). Such molecular mobility confers on serine proteinase inhibitors, the capacity not only to bind and to entrap their target proteinases in the highly stable complexes, but also a concomitant propensity to form dysfunctional molecules. For example, natural mutations either alone or in combination with environmental influence, can block flexibility in the structure that is necessary for the normal inhibitory activity. These mutations also promote serpin self-assembly and depletion of the active serpin activity. On the other hand, these self-assembled abnormal serpins themselves can directly or indirectly lead to pathological diseases. Several well-characterized examples include the emphysema (serpin, α1-antitrypsin depletion) [58], liver cirrhosis (α1-antitrypsin intracellular inclusions) [48], and the dementia associated with neuroserpin inclusion bodies [12]. The loss of inhibitory activity of serpin results in the deregulation of the delicate balances between proteases and anti-protease activities. Under some conditions the critical balance between native form of serpin may be significantly tilted to the conformationally altered forms. Generation of a large fraction of these new molecular forms with biological activities unrelated to inhibition of protease

activity, may not only induce imbalance in protease and antiprotease system, but also may promote other physiological or pathological processes.

3. Serpins in neurodedegenerative brain diseases

Interests in serine and cysteine proteinases and their inhibitors as well as their roles in the pathogenesis of neurodegenerative diseases expanded recently, largely because these proteinases and their inhibitors were detected at sites of brain injury [22,46,56]. Moreover, several proteases have been found to play multiple roles in central nervous system. For example, thrombin is shown to protect astrocytes against hypoglycemia or oxidative stress, but also found to increase neuronal death; tissue type plasminogen activator (tPA) protects neurons against Zn-induced cell death, but also found to mediates neuronal death induced by excitotoxins [17,44]. It is inferred from different studies that expression of serine proteinases is increased during the brain injury that leads to impairment in the balance between serine proteinases and their cognate inhibitors in the brain, which may eventually lead to a pathologic state. Therefore, the levels of native and modified forms of serpins are a critical factor in understanding neurodegenerative conditions. The expression of the serpins like Type-1 plasminogen activator inhibitor (PAI-1) and protease nexin-1 (PN-1) is increased in the cerebrospinal fluid from patients with neurologic disorders such as Alzheimer's disease, cerebral infarction, infection and neoplasia [17,56]. Increased levels of antithrombin, an inhibitor of the blood coagulation cascade, and alpha-1-antichymotrypsin (ACT), an inhibitor of proteinases of the chymotrypsin class, are also found in Alzheimer's disease patients [2,63].

Different studies suggest that serpin levels might influence the outcome of brain injury in that is unrelated to their property to inhibit over-expressed protease activity. For example, PAI-1 produced by astrocytes is found to be neuroprotective against necrosis mediated N-methyl-D-aspartate [6]. PN-1 is reported to protect cultured neurons from glucose deprivation-induced damage through attenuation of the increase in intracellular calcium levels associated with such damage [56]. Neuroserpin is a newly identified member of the serpin family that is primarily expressed in brain, and primarily localized to neurons [46]. 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 [45]. Neuroserpin is also suggested to be an important factor contributing to neuronal plasticity and learning [53]. Mutations in the neuroserpin gene result in polymerization and aggregation of the neuroserpin protein, with consequent diminution in neuroserpin levels that might result in uncontrolled proteolysis and the loss of neuronal function. On the other hand, it is equally conceivable that the inclusion bodies formed from the aggregated neuroserpin, so-called Collins bodies [12] are neurotoxic. This provides evidence that the inclusion bodies themselves may have pathological effects.

4. Multiple biological activities of α1-antichymotrypsin

Alpha-1-antichymotrypsin (ACT) is a glycoprotein with a molecular weight of 55–68 kDa and 25% by weight of various sugars, whose heterogeneity results in variation of molecular weight. The gene encoding α1-antichymotrypsin is found on the distal region of the long arm of chromosome 14 (14q32) [28]. The normal adult plasma α1-antichymotrypsin concentration ranges between 0.3–0.6 mg/ml. α1-antichymotrypsin is an acute phase protein and plasma α1-antichymotrypsin levels might increase rapidly more than 5-fold during an acute phase reaction [26]. α1-antichymotrypsin is synthesised primarily by hepatocytes and secreted into blood plasma. The expression of α1-antichymotrypsin in hepatic cells is known to be enhanced by interleukin-6 (IL-6), to some extent by IL-1, and by glucocorticoids [11,30,31, 43]. Although most protease inhibitors, including α1-antichymotrypsin, are found in plasma as a circulating proteins released from hepatocytes, the biosynthesis of these inhibitors has also been demonstrated in extrahepatic sites. Synthesis of α1-antichymotrypsin has been reported in human bronchial and breast epithelial cells, in the epididymal cells, particularly those of the choroid plexus in normal brain [4,28], in activated astrocytes and to a small extent in monocytes. Brain microvessel endothelium cells also release α1-antichymotrypsin [1, 2,21], thus suggesting that the inhibitor may perform unique functions in local microenvironments.

Although much is known about the biochemistry of α1-antichymotrypsin, its physiological role is largely unknown. α1-antichymotrypsin appears to specifically inactivate serine proteases such as neutrophil cathepsin G, mast cell chymase and pancreatic chymotrypsin through the formation of a complex between α1-antichymotrypsin and protease. In addition, the ma-

jor fraction of human prostate derived proteases belonging to the kallikrein family of enzymes used clinically to monitor patients with prostate cancer, are found in complex with α1-antichymotrypsin [57]. Various complexes between α1-antichymotrypsin and proteases are known then bind back to cell surface receptors with eventual internalisation by the cell.

Interestingly, α1-antichymotrypsin is only known serpin to display DNA binding activity which is completely independent from its protease inhibitory activity [37,42]. It has been postulated that α1-antichymotrypsin's DNA binding capability may provide a mechanism for the self-regulation of its own gene expression. It was reported that α1-antichymotrypsin inhibits DNA synthesis in permeabilized human carcinoma cells which suggests that serpin may inhibit DNA polymerase and/or DNA primase [59,61].

Alpha-1-antichymotrypsin may also be involved in controlling oxidative damage because of its correlation of inhibition of oxygen consumption and superoxide generation in human granulocytes. Complexes formed by α1-antichymotrypsin and chymotrypsin have been shown to regulate superoxide production in neutrophil membranes by interacting with NADHP oxidase [29].

The fact, that α-antichymotrypsin has been shown to inhibit proteases and to control superoxide generation show this protein to be highly anti-inflammatory and play a role in defense mechanisms in various pathological processes. Indeed, the induction of expression of α-antichymotrypsin during inflammatory processes might stimulate α-antichymotrypsin interaction not only with over expressed target proteases but also with other molecules [24,42]. The property of α-antichymotrypsin under certain circumstances to interact with other molecules such as DNA, non-target proteases and peptides may result in loss of inhibitory activity of α-antichymotrypsin as well as in occurrence of the new molecular forms of α-antichymotrypsin having different biochemical properties and physiological roles compared to native α-antichymotrypsin.

5. Correlation of α-antichymotrypsin and Alzheimer's disease

Alzheimer's disease is an age-related, irreversible brain disorder that occurs gradually and results in memory loss, behaviour and personality changes, and a decline in thinking abilities. Scientists estimate that up to 20 million people worldwide currently suffer with this disease, and the prevalence (the number of people with the disease at any one time) doubles every 5 years beyond age 65. It is also estimated that approximately 360,000 new cases (incidence) will occur each year and that this number will increase as the population ages.

The causes of this disease are not known, but major risk factors include old age and a family history of dementia, Down's syndrome, female gender, low level of education, and head injury. The disease is characterized by abnormal accumulation of amyloid-beta (Aβ) peptide, the protein Tau and other inflammation related proteins such as alpha1-antichymotrypsin in the extracellular space and nerve cells, respectively of certain regions of the brain [5,33,36]. A link between Alzheimer's disease and α1-antichymotrypsin was established through the observations that α1-antichymotrypsin is specifically found both in amorphous and classic plaques of Alzheimer's disease, and in amyloid deposits in Down Syndrome and normally aged brains [2,49]. These observations suggest that α1-antichymotrypsin may play rather specific role in Alzheimer's disease pathogenesis.

Much of interest in Alzheimer's disease is focused on the role of the proteases and their inhibitors. There is a very delicate balance in the development and remodeling of tissue structures. The initial suggestion that Alzheimer's disease might be a result of imbalance in protease and anti-protease system in the brain originated from the several independent studies. The main component of Alzheimer plaques, Aβ, is proteolytically derived peptide from the larger precursor protein [10,15]. α1-antichymotrypsin is not only found to colocalize specifically with Aβ_{1-42} deposits, but also its expression is increased in areas of brain that is prone to plaque development [30]. These accumulated observations further strengthened the significance of protease and anti-protease balance in the pathogenesis of Alzheimer's disease.

5.1. α-antichymotrypsin and inflammation in the brain

The role of α1-antichymotrypsin in the brain of Alzheimer patients and biochemical pathways controlled by α1-antichymotrypsin is still under active investigations. Several unexpected functions for α1-antichymotrypsin were shown in addition to its known protease inhibitory activity. For example, α1-antichymotrypsin was shown to be active against extracellular proteins such as proteoglycans that are also present in AD plaque [33]. Presence of inflammatory components, such as complement factors, amyloid P

component, α-2-macroglobulin, proteoglycans and α1-antichymotrypsin in senile plaques, suggested a possible function for α1-antichymotrypsin in an acute phase response. Experiments in human astrocyte cultures have shown that interlukine I (IL-1) induces astrocytic expression of α1-antichymotrypsin [11]. Interestingly, α1-antichymotrypsin has been shown to upregulate astrocytic production of IL-1 [41]. Thus, there seems to be a positive feedback self-regulation circuitry.

5.2. *α1-antichymotrypsin and oxidative damage*

The α1-antichymotrypsin has also been implied to be involved in controlling oxidative damage since it has been shown to inhibit oxygen consumption and superoxide generation in human granulocytes [29]. Moreover, an increased serum α1-antichymotrypsin has been found to associate with an increased capacity for generating superoxide derivatives in neutrophils from the peripheral blood of Alzheimer's disease patients [34]. These findings suggest that α1-antichymotrypsin may play a functional role in oxygen radical metabolism. α1-antichymotrypsin may also involve in oxidative stress in the brain of Alzheimer's patients because there is a correlation between the elevated α1-antichymotrypsin levels and an increased oxidative damage in the central nervous system of these patients [21].

5.3. *α1-antichymotrypsin and a complex formation with Aβ_{1-42}*

A striking property of α1-antichymotrypsin is its ability to bind Aβ in a simulated mode [25,26]. α1-antichymotrypsin similar to other serpins is an irreversible, suicide inhibitor of proteases with a positionally conserved at reactive site that acts as a "bait" for a serine protease. Proteolytic cleavage of the reactive center loop by proteases leads to insertion of the strand N-terminal to the cleavage site as strand 4 into β-sheet A [63]. A large α-sheet A can also be opened to accept exogenous peptides of sequences similar to that of the reactive site loop [52]. Several investigators including one of us (S.J.) have shown experimentally that α1-antichymotrypsin not only forms stable complexes with Aβ_{1-42}, comparable in specificity and stability to a proteinase-inhibitor interaction, but also it can inhibit or accelerate fibril formation of Aβ_{1-42} in a concentration dependent manner [24]. Furthermore, complexes Aβ_{1-42} with α1-antichymotrypsin significantly deplete the available α1-antichymotrypsin, therefore render proteinase inhibitory activity. α1-antichymotrypsin also accelerates polymerization of Aβ_{1-42} to its fibrillar form and itself polymerizes spontaneously [14,17, 36].

Thus, α1-antichymotrypsin has been found to indirectly act as a molecular chaperone to influence fibril formation through specific complexes with Aβ_{1-42}. Likewise, Aβ_{1-42} reciprocally abolishes α1-antichymotrypsin natural inhibitor activity. Depletion of α1-antichymotrypsin molecules could lead to unregulated protease activity associated with Aβ_{1-42} biosynthesis, cytokine activation or other physiological consequences. It was recently shown that α1-antichymotrypsin can indirectly inhibit a proteinas that is responsible to degrade Aβ_{1-42} [39,65]. These studies suggest that serine proteinases and their inhibitors are extensively involved in inflammatory processes.

5.4. *α1-antichymotrypsin and the Alzheimer's disease*

The complex of Aβ_{1-42} with α1-antichymotrypsin structurally resembles that of serpin/proteinase complexes [25]. Since the latter have biological activities beyond that of proteinase inhibition such as neutrophil chemoattractant activity and up-regulates synthesis of own serpin, it is possible that α1-antichymotrypsin/Aβ_{1-42} complexes also have as yet other uncovered biological activities which may contribute to the establishment of the self-propagating neurotoxic pathologies. α1-antichymotrypsin/proteinase complexes and/or α1-antichymotrypsin/Aβ_{1-42} complex may feedback up-regulate α1-antichymotrypsin biosynthesis through raising local α1-antichymotrypsin levels in the presence of increasing Aβ_{1-42}. Thus it could sustain pathological cycles.

The accumulated evidence supports that α1-antichymotrypsin promotes rather than inhibits the development of Alzheimer's disease. Recently it has been demonstrated in an amyloid precursor protein transgenic mouse model that human α1-antichymotrypsin increases the age-dependent accumulation of cerebral amyloid plaque [41]. Astroglial overproduction of human α1-antichymotrypsin was found to be amyloidogenic *in vivo* [1]. Furthermore, the brain α1-antichymotrypsin level correlates with the number of activated astrocytes [41] indirectly support these findings. Increased levels of α1-antichymotrypsin, of which a larger fraction is polymeric, are found in plasma and cerebrospinal fluid samples from Alzheimer's disease patients [50]. Other observations showing that the plasma levels of α1-antichymotrypsin

in Alzheimer's disease patients correlates well with the degree of cognitive deterioration as assessed by the MMSE or Global Deterioration Scale [34,35,40].

6. Beyond serpins

Recent studies revealed that Aβ can be neurotoxic by a mechanism(s) involving free radical and inflammatory molecular production and loss of cellular ion homeostasis [19]. The physical state of Aβ has long been considered a determinant of its neurotoxicity, with the fibrillar form inferred to be neurotoxic [20,54]. Aβ, however, can form small soluble oligomers under conditions that block fibril formation, for example during selective interaction of Aβ with other molecules, including proteins found in neuritic plaques [5,68]. These oligomers were found to be highly neurotoxic [32], although the basis for their potent toxicity remains unknown. On the other hand, physiological levels of Aβ peptides are shown to promote the growth of neuroblastoma cells in low serum medium to twice the rate [38], suggesting that Aβ may normally play a neurotrophic/mitogenic role in neuronal biology. These seemingly dissipated findings together clearly show that Aβ, depending on its molecular form, may play the multiple-patho-physiological roles.

A yeast protein zuotin has also been found to be able to perform multitasks. It was initially identified for its preferential ability to bind to left-handed Z-DNA [67] and was later found that zuotin was also involved in tRNA transport from nucleus to the cytoplasm [64], and possesses chaperon function assisting in protein folding [66].

It has also been reported that tyrosyl-RNA synthetase can be split into two fragments with distinct cytokine activities [62]. Several house-keeping proteins have been implicated to have multiple functions. Those include PutA proline dehydrogenase, phosphoglucose isomerase, thymidine phosphorylase, carbinolamine dehydrogenase, thrombin protease, E.coli thioredoxin, uracil-DNA glycosylase, thymidine synthetase and others. With the avalanche amount of protein information from the genomic and proteomic activities, we can expect to find the common phenomena of multitask capability of proteins and to uncover their secret of life.

7. Conclusion remarks and perspectives

Look back just 50 years ago, if someone suggested that a gene could code for more than one protein, it would be considered to be radical. The prevailing wisdom then was that one gene coded one enzyme. Who could then have imaged that a single gene could code for several proteins through alternative RNA splicing [18] and protein splicing [47]? We should have an open mind and expect the unexpected multitask capability of proteins in the coming years.

Mozart is undoubtedly one of the most capable multitasking musician ever lived. He played, composed for numerous kinds of instruments, and conducted a diverse type of music and opera. Can proteins posses such multitask talents? Stay tuned!

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