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*Critical Review*

# Serpin Polymerization and Its Role in Disease—The Molecular Basis of $\alpha_1$-Antitrypsin Deficiency

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***Summary***

**Protein aggregation is the cause of several human diseases. Understanding the molecular mechanisms involved in protein aggregation requires knowledge of the kinetics and structures populated during the reaction. Arguably, the best structurally characterized misfolding reaction is that of $\alpha_1$-antitrypsin. $\alpha_1$-Antitrypsin misfolding leads to both liver disease and emphysema and affect approximately 1 in 2000 of the population. This review will focus on the mechanism of $\alpha_1$-antitrypsin misfolding and the development of potential therapeutic strategies.** 

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## INTRODUCTION

There is an increasing number of diseases, reaching from early onset metabolism disorders to late onset neurodegenerative diseases, where inappropriate conformational change leads to the adoption of a species which self-associates (*1*). If this self-association continues unhindered then aggregates are formed which can deposit within and outside the cell. Recently, it has become clear that it is the species formed early in this reaction which are the underlying cause of toxicity. In all protein misfolding diseases self-association occurs by $\beta$-sheet linkages which can result in either amyloid or polymeric structures (*2*) (Fig. 1). Structural characterization of the species formed during aggregation is technically challenging. Arguably, the best structurally characterized aggregation reaction is that of $\alpha_1$-antitrypsin ($\alpha_1$AT) where structures of the initial (*4*) and aggregated forms (*5, 6*) are known in crystallographic detail. $\alpha_1$AT is a member of the serpin superfamily and its polymerization leads to both emphysema and severe liver disease. In this review we describe the kinetic mechanism and structural changes involved in $\alpha_1$AT polymerization and review potential therapeutic options.

## THE SERPIN SUPERFAMILY: STRUCTURE AND FUNCTION

The serpin superfamily is composed of a vast variety of proteins with diverse functions that all share a conserved core domain of about 400 residues (*7*). The family name was originally chosen in accordance with the fact that most serpins initially identified were serine proteinase inhibitors (*8*). However, the serpin superfamily also includes cysteine proteinase inhibitors (*9*), dual class inhibitors that have both serine and cysteine proteinase inhibitory activity (*10*) as well as noninhibitory serpins (*11*). Approximately 1,500 serpins have been identified so far with members having key roles in the coagulation, inflammatory, and fibrinolytic cascades.

Despite relatively low sequence identity amongst family members, the secondary and tertiary structures of all serpins structurally characterized to date show significant similarities within the core domain. This core domain consists of three $\beta$-sheets (A–C) and nine $\alpha$-helices (A–I) (*12*). In the native state the central five stranded $\beta$-sheet A supports an exposed reactive center loop (RCL) that extends to $\beta$-sheet C (Fig. 2). In inhibitory serpins the RCL contains the cleavage site of the target proteinase. Upon proteolytic cleavage the RCL inserts into the middle of the central $\beta$-sheet A as a new strand (s4A) thereby creating a six stranded $\beta$-sheet A. During this conformational rearrangement the proteinase, which is covalently attached to the RCL, is dragged from the proximal to the distal end of the serpin (*13*). This translocation leads to a change in structure and the irreversible deactivation of both the serpin and the proteinase, hence the term suicide-substrate mechanism (*14, 15*).

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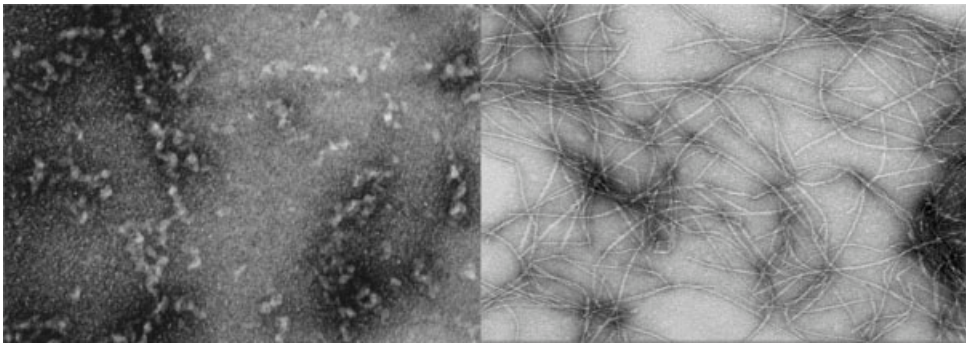

**Figure 1.** Electron micrographs of protein aggregates. The panel on the left shows $\alpha_1$AT polymers; the panel on the right shows fibrils formed by the polyglutamine repeat protein ataxin-3 (*3*).

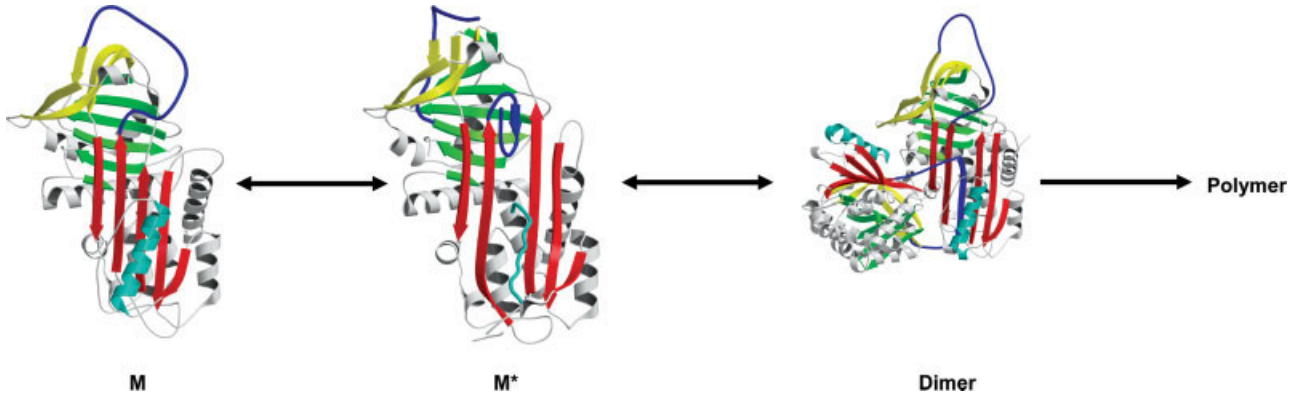


**Figure 2.** Mechanism of $\alpha_1$AT polymerization. M represents native $\alpha_1$AT with $\beta$-sheet A in red, $\beta$-sheet B in green, $\beta$-sheet C in yellow, the RCL in dark blue, and the F-helix in light blue (pdb identifier: 1QLP). M* is formed by unfolding of M which disrupts $\beta$-sheet A and the F-helix. M* is a polymerogenic species that self-associates to give rise to dimers which irreversibly further associate forming the mature polymers.

## METASTABILITY AND ITS ROLE IN SERPIN MISFOLDING

Considerable structural flexibility within the serpin is required to accomplish the conformational change essential for proteinase inhibition (*14*). The energy for this transition is derived from the serpin's metastable native fold: serpins do not fold to their thermodynamically most favorable conformation. RCL insertion into $\beta$-sheet A results in a significant decrease in free energy and is therefore a thermodynamically favorable process. Thus, the serpin's native state is a kinetically trapped folding intermediate and the driving force during proteinase inhibition is the change in free energy upon RCL cleavage resulting in the more stable cleaved serpin (*16, 17*).

The inherent structural flexibility of inhibitory serpins coupled with the metastable native state render the serpin architecture vulnerable to mutations that result in dysfunctional folds. In some cases mutations lead to the formation of inactive serpin conformations with increased stability relative to the wild type. These high stability conformations all involve at least a partial insertion of the RCL into the serpin either intramolecular forming the latent or the delta conformations (*18*) or intermolecular leading to serpin polymerization (*19–21*). Even though the latent and the delta conformations are implicated in disease serpin polymers reflect the most prominent of these alternative high stability states as they are the cause of most serpinopathies (*22*). Serpin polymerization occurs when the RCL of one molecule is inserted into a $\beta$-sheet of another molecule to then form further intermolecular linkages. Loop A-sheet polymers, which arise through the insertion of the RCL of one molecule into $\beta$-sheet A of another, thus becoming strand 4 (s4A), are pathologically the most relevant as they can form under physiological conditions (*19*) (Fig. 2).

## SERPIN FOLDING, MISFOLDING, AND POLYMERIZATION

A number of folding studies have revealed that serpins fold to their native conformation via at least one intermediate en-

semble (*23–27*). This ensemble is characterized by the presence of an intact $\beta$-sheet B, partially folded $\beta$-sheet A and C (*23*) and a grossly disrupted F helix (*28, 29*). Recently, evidence has been presented which suggests that this intermediate ensemble has many of the properties of a molten globule (*30*). Extensive non-native interactions are formed while folding which are proposed to prevent misfolding by restricting the conformational space available to the folding molecule (*22*). Disruption of key interactions within the intermediate ensemble results in the transition of the folding serpin molecule to a polymeric state.

Serpin polymerization consists of at least two steps (*31*) (Fig. 2). First, the native state (M) undergoes a conformational change to a polymerogenic monomeric species termed M* (*32, 33*). M* then self-associates to form dimers (*34*) that can either dissociate to monomers or continue self-associating to irreversibly form long chain polymers (P). According to this kinetic scheme the formation of P is dependent on the concentration of M* and therefore the rate of M* formation. Mutations that either lead to a destabilization of the native fold and hence favor the formation of M* or that decrease the kinetic barrier to attain M* will result in an increased polymerization rate and disease.

## $\alpha_1$ANTITRYPSIN DEFICIENCY

The most common serpin-related misfolding disease is $\alpha_1$AT deficiency which affects approximately 1 in 2000 people (*31, 35*). $\alpha_1$AT is an acute phase glycoprotein that is synthesized and secreted from the liver. Its major physiological role is to inhibit neutrophil elastase (NE) in the lung interstitium. NE is a serine proteinase involved in the inflammatory response and its uncontrolled activity leads to parenchymal lung destruction as it degrades several connective tissue constituents. Since $\alpha_1$AT is the major inhibitor of NE, a significant decrease of $\alpha_1$AT activity leads to tissue destruction and emphysema due to a protease-antiprotease imbalance (*35*).

Several disease-causing variants of $\alpha_1$AT have been identified including Z (E342K), S (E264V), and null variants with the Z variant being responsible for 95% of $\alpha_1$AT deficiency cases. The Z mutation results in an increased propensity of $\alpha_1$AT to polymerize in the endoplasmic reticulum of hepatocytes leading to a lack of secretion into the circulation (*31*). The Z $\alpha_1$AT phenotype is characterized by a reduction in plasma levels to 10–15% of normal. This correlates inversely with an increased risk of both emphysema and severe liver disease. Liver disease occurs in $\sim$15% of cases of $\alpha_1$AT deficiency and is associated with an accumulation of the polymerized protein within hepatocytes in combination with other genetic traits and/or environmental factors (*36*).

The Z mutation is located at the top of strand five of $\beta$-sheet A and the base of the RCL. In $\alpha_1$AT a glutamate residue at position 342 forms a hydrogen bond with threonine 203 and a salt bridge to lysine 290. The Z mutation leads to the disruption of these interactions causing a conformational change around the top of $\beta$-sheet A which is proposed to make it more receptive to the RCL of another molecule. Therefore, the Z mutation promotes loop A-sheet polymerization of $\alpha_1$AT (*32*).

The mechanism by which the Z mutation results in misfolding is not well defined. Controversy exists on whether it leads to a destabilization of the native state or to a reduction in the kinetic barriers to polymer formation. A study conducted by Dafforn et al. (*32*) showed a decreased thermodynamic stability of Z $\alpha_1$AT as assessed by thermal denaturation suggesting an increase in M* and hence the formation of polymers at lower activation temperatures compared to the wild type. Controversially, a study by Yu et al. found that the stability of Z $\alpha_1$AT appeared to be similar to that of wild type $\alpha_1$AT as estimated by unfolding in urea gradient gels (*37*). The latter study implied that the Z mutation does not lead to a drastic destabilization of the native fold of $\alpha_1$AT. Although there is no kinetic data to support this theory, the authors concluded from their study that the transition rate from the intermediate to the native state is altered for Z $\alpha_1$AT. This would lead to an accumulation of M* and hence increased polymer formation.

Several intriguing questions regarding Z $\alpha_1$AT and potential therapeutic strategies remain to be solved: Does the Z mutation lead to an altered thermodynamic stability of the native state of $\alpha_1$AT and/or different folding kinetics? If Z $\alpha_1$AT polymerizes via loop A-sheet polymers is it possible to stabilize the monomeric inhibitory active serpin? What other factors besides Z $\alpha_1$AT aggregation play a role in the establishment of $\alpha_1$AT deficiency associated liver disease as it occurs in only a minority of patients?

## POTENTIAL THERAPEUTIC APPROACHES

Most current therapeutic approaches in the treatment of $\alpha_1$AT deficiency are directed toward the prevention of pulmonary tissue destruction. In the augmentation therapy $\alpha_1$AT is administrated either intravenously or as an aerosol to raise/maintain $\alpha_1$AT serum levels above the critical threshold for disease. No specific therapeutic strategy has been developed for $\alpha_1$AT deficiency associated liver disease and often liver transplantation presents as the only solution.

Current research is focused on the development of novel therapeutic strategies that target the molecular basis of the disease. In regard to liver disease in $\alpha_1$AT deficiency, it is therefore essential to identify additional genetic factors that are involved in the abnormal phenotype such as faults in the quality control system. Such essential pathways could present possible pharmacological targets for increasing the rate of aggregate clearance hence decreasing cytotoxicity. A potential therapeutic strategy in the treatment of $\alpha_1$AT and other diseases caused by abnormal protein folding and mislocalization may involve the utilization of chemical chaperones that facilitate folding to the active native conformation (*38–40*). The

application of 4-phenylbutyric acid in a model cell culture system, for instance, has already shown to increase the secretion of active Z $\alpha_1$AT (*36*).

Because polymerization and aggregation of Z $\alpha_1$AT forms the molecular basis of most $\alpha_1$AT deficiency cases, the prevention of polymer formation and the stabilization of the native state could have great potential as a therapeutic strategy. *In vitro* studies utilizing peptides homologous to the RCL of $\alpha_1$AT have shown that they can bind to $\beta$-sheet A and block polymerization (*36*). The identification of similar stabilizing peptides or molecules may also provide a strategy in the treatment of other serpinopathies that are based on polymerization. It is therefore essential to identify and characterize the precursors to these misfolded serpins to allow the development of polymerization inhibitors that preferably stabilize the serpin's active monomeric fold.

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