

HISTORICAL REVIEW

What We Owe to α_1 -Antitrypsin and to Carl-Bertil Laurell

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ABSTRACT

The archetypal status of α_1 -antitrypsin in biology and medicine grew from the finding, thirty years ago, by Carl-Bertil Laurell, of the association of its deficiency with emphysema. In biology, α_1 -antitrypsin now provides the model for both the structure and the remarkable mechanism of the serpin protease inhibitors that control the key proteolytic pathways of the body. In medicine, the plasma deficiency of α_1 -antitrypsin has drawn attention to protease-antiprotease imbalance as a contributory cause of chronic obstructive pulmonary disease. But even more significantly, the finding that the common genetic deficiency of α_1 -antitrypsin was also associated with the development of liver cirrhosis introduced the new entity of the conformational diseases. The proposal that the same general mechanism was responsible for the best known of the conformational diseases, the common late-onset dementias, was controversial. It was vindicated however by the recent finding that a mutation, which results in the liver aggregation of α_1 -antitrypsin, also results in a typical late-onset dementia when it occurs in a brain-specific homologue of α_1 -antitrypsin. The extensive development of such diverse fields of studies, each based on α_1 -antitrypsin, is a measure of the encouragement Laurell gave to younger colleagues in the field. It also reflects the great advantage of linked contributions from clinical as well as basic sciences. Time after time, scientific controversies and deadlocks have been solved by landmark clinical cases, which have revealed unexpected findings and insights, within and beyond the fields of study.

Key Words: Serpin; α_1 -antitrypsin; Emphysema; Conformational disease; Cirrhosis; Laurell; Carl-Bertil.

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INTRODUCTION

The unique contribution of clinical medicine to biological science is its ability to provide totally unexpected leads that open new horizons in understanding and research. Such was the case 30 years ago (1) when the Swedish medical biochemist, Carl-Bertil Laurell, observed the absence of the alpha-1 protein band in plasma from two patients in a respiratory-disease hospital (Figure 1). The change in the electrophoretic pattern was quickly identified as being due to a deficiency of a plasma protease inhibitor of previously uncertain significance (2), called α_1 -antitrypsin. This finding of the deficiency of a protease inhibitor not only revealed a prime mechanism underlying the development of emphysema (1,3), but also gave a handle that led to the identification of the function and dysfunctions of a whole new family of protease inhibitors—the serpins (4–6). The special significance of this family to medicine (7) is that its members, all of which bear a close identity in structure and function to that of α_1 -antitrypsin (8), control the crucial proteolytic cascades of the plasma. So the impetus provided by Laurell's observation has directly contributed to the understanding we now have of the way antithrombin controls coagulation, of how heparin modulates this activity and of how mutations in antithrombin result in familial thrombosis. Likewise we can now see how the plasminogen activator inhibitor PAI-1 controls fibrinolysis, how C1-inhibitor controls complement activation and similarly how other intracellular as well as extracellular pathways are controlled by the many other members of the serpin family that have now been

identified. But the special bonus from Laurell's discovery was the later realisation that the deficiency of α_1 -antitrypsin was due to its misfolding and aggregation at its site of synthesis in the liver. The way in which this aggregation in the hepatocytes leads on to liver damage and eventual cirrhosis provided the first detailed model for a whole new category of disorders—the conformational diseases (9). With this came the implication that the same process, affecting other proteins, would underlie the common dementias such as Alzheimer's and Parkinson's diseases. This prediction was vindicated by the recent finding, in a similar late-onset dementia, of mutations in a brain-specific serpin, which are identical in nature and consequence to those in α_1 -antitrypsin resulting in cirrhosis (10,11). The universality of Laurell's finding to biology as a whole is highlighted by the most recent finding: that a disease phenotype in the fruit-fly *Drosophila* (12) results from a mutation precisely identical to that causing the common deficiency of α_1 -antitrypsin in people of European descent. The story of the way all of these understandings developed from the original seminal observation by Laurell, is a fascinating one. It is told here from the perspective of a participant in the story who viewed much of the events from opposite poles of the earth—in New Zealand till 1986 and after that in Cambridge, England.

FINDING OF THE DEFICIENCY

Serendipity leads to discovery when it presents to a receptive and prepared mind. Laurell was 42 years old when he first observed the deficiency of α_1 -antitrypsin in 1962. By this time he had already made distinguished contributions to protein research, with the finding and naming in his doctoral thesis of transferrin (13), and subsequently with the recognition of caeruloplasmin (14) and the defining of the function of haptoglobin (15). Technically, his outstanding contribution was the use of plasma protein electrophoresis as a tool for clinical investigations. This originated with the use of paper electrophoresis following his visit to the laboratory of Tiselius in 1952 and then, in 1961, in the introduction of the much more selective agarose gel electrophoresis (16). The power of these techniques is well illustrated by their usage by a haematological physician, J Waldenström, whose office was adjacent to Laurell's laboratory in Malmö. Together they described (17) the finding of 'M' proteins in myeloma leading to the recognition of the eponymously named Waldenström's macroglobulinaemia. Other medical investigators in Malmö took advantage

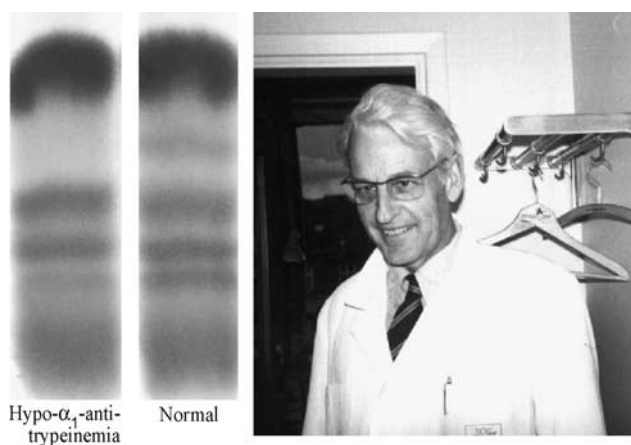


Figure 1. C-B Laurell (1919–2001). Photographed in Malmö in 1972. Left, the original paper electrophoretic strips from 1962 showing the almost complete absence of the alpha-1 band from the plasma of a respiratory disease patient.



of the new tool of electrophoresis and in 1961 a newly appointed senior respiratory physician introduced its routine use for all his patients. Laurell personally checked every electrophoretic result and noted the absence of the alpha-1 band in two samples, both of which had originated from the specialist respiratory hospital. Laurell then contacted a young physician-investigator, Sten Eriksson, to take part in a joint clinical laboratory survey. Eriksson did this with enthusiasm and thoroughness. Two further examples were found on a survey of past electrophoretic strips and then two further new examples were identified. The critical finding was that four of these first six cases of alpha 1 deficiency had emphysema. Laurell and Eriksson published their findings (1,3) in 1963, with Eriksson giving the first comprehensive account of the clinical syndrome associated with the electrophoretic deficiency, in his thesis of 1965 (18).

The achievements of Eriksson underline a special strength of Carl-Bertil Laurell, in not only being able to inspire others but also in providing an initial impetus to their work and then stepping aside. The next decade was a time of extraordinary productivity in Malmö. Ohlsson and Ganrot and then Jeppsson joined Laurell in the biochemical investigation of the deficiency of α_1 -antitrypsin. In addition two early clinical studies were published that are landmarks in the field—Larsson provided what is still the definitive description (19) of the interaction of smoking with the deficiency and Sveger commenced the study of affected newborns that now defines the natural history of the syndrome (20). Young investigators from abroad also came to Malmö (Figure 2), and two of these, Magne Fagerhöl from Norway and Diane Cox from Canada, led the work that clarified the complexities of the genetic variants of α_1 -antitrypsin (21,22). It is necessary to recall now the difficulties they faced. In the 1960's haemoglobin provided the only defined molecular model for protein variation in human blood. But unlike haemoglobin, α_1 -antitrypsin is a glycoprotein and as such moves as a series of bands on electrophoresis quite unlike the clear single change in mobility observed with the abnormal haemoglobins. So in the first years following the discovery of the deficiency of α_1 -antitrypsin it was thought that the underlying abnormality was one of glycosylation, rather than of a single amino acid substitution as seen with Hb S and the other variants of haemoglobin. Furthermore, although as we now know, the Z variant of α_1 -antitrypsin is responsible for its severe deficiency, the European also carries, at even greater frequency, the milder deficiency S variant. Fagerhöl and Cox systematically defined the different genetic variants

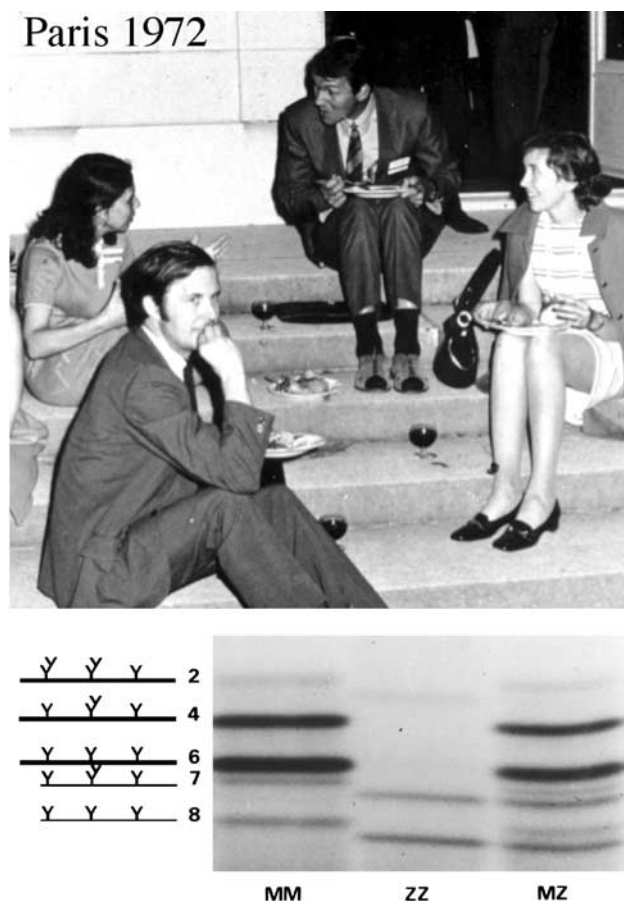


Figure 2. Laurell's initial finding soon inspired international research. Here at the Congress of Human Genetics in Paris in 1972 are, on the right Magne Fagerhol from Norway and Diane Cox from Canada, and in the foreground the author from New Zealand. The need at that time was to explain the complex electrophoretic banding of antitrypsin (below) which was categorised by Fagerhol and Cox with the Pi nomenclature (22) and later shown (109) with Jeppsson (50) and others (66,110), to be due to glycoforms and post-translational changes as depicted to left.

and their frequencies of occurrence by introducing the Pi system of electrophoretic analysis (23,24).

THE ALPHA-1 SYNDROME

The recent suggestion by John Humphries, Robert Stockley and others, that the clinical manifestations accompanying the common homozygous Z state be referred to as a syndrome rather than a deficiency, deserves encouragement. It is too late to change the name of α_1 -antitrypsin itself, which although a mouthful and a misnomer (it will be also referred to

here as antitrypsin), has become irrevocably established in the literature. But the description of the clinical abnormality as 'α₁-antitrypsin deficiency' continues to be misleading as well as confusing. Although the plasma deficiency of the Z homozygote is directly related to the predisposition to obstructive lung disease, the label of α₁-antitrypsin deficiency obscures what is a major pathological consequence of the ZZ state—a variably progressive loss of hepatocytes with the potential development of liver cirrhosis (25). The early studies of the Malmö group noted the relationship of α₁-antitrypsin deficiency with liver disease but the essential link between the two became apparent with the report in 1971 by a US paediatrician, Harvey Sharp (26), of the presence of inclusions of antitrypsin in the livers of children, homozygous for the Z mutation, who developed a rapidly progressive cirrhosis (27). Once again it was studies from Malmö that put this finding into context. Sveger, in his comprehensive study of newborn Z homozygotes (20,28), showed that near 10% developed a neonatal cholestasis with progression, in some 1–2% overall, to a juvenile cirrhosis. This tragic but relatively uncommon consequence of Z homozygosity overshadowed the much more frequent occurrence of a slowly progressive portal fibrosis, which in some 20% of homozygotes results in cirrhosis in later adult life (19,29). A key contribution to fitting all these findings together, within the framework of the molecular pathology of the alpha-1 syndrome, was made by Jeppsson (30) who showed that the hepatocyte inclusions (31), which characterised the liver disease, were solely formed of the intact variant Z protein.

The original observation of the association of the plasma deficiency of α₁-antitrypsin with emphysema provided an immediate stimulus to research into mechanisms of obstructive lung disease. Although this was initiated in Sweden (32) much of the activity was in the US, and in particular from groups with specialist respiratory interests including those of Lieberman (33), Janoff (34), Pierce (35), Senior (36) Snider (37) and Crystal (38,39). Their work established the role of antitrypsin as an inhibitor of the elastase released by activated leukocytes. From this came the concept of the protease-antiprotease balance as an essential requirement for respiratory health, with perturbations of this balance resulting in the progressive loss of lung elasticity. The findings opened insights into the contribution of proteolytic overload to the much more common occurrence of emphysema due to tobacco smoking. Biochemical and cellular studies (40–44) studies showed the vulnerability of α₁-antitrypsin to oxidative inactivation (though disappointingly there has been little recent follow-up of these findings of two decades ago). The predictable additive effect of smoking

in conjunction with a plasma deficiency antitrypsin, fitted with the earlier findings of Larsson (19) and others (45) showing a catastrophic acceleration of the age of onset of emphysema in Z homozygotes who were also current or ex-smokers.

THE S AND Z VARIANTS

A theme of this review is the way an original seminal finding is disseminated and inspires the work of others. This dissemination occurs through multiple interactions that are appropriately illustrated at this stage if the author tells the story of his own involvement. This dates from my doctoral studies, on the molecular pathology of haemoglobin, in the Biochemistry Department of the University of Cambridge. With the completion of this work in 1967, came an invitation to speak at a British Council course for young investigators from Europe. After the talk I was approached by one of the participants in the course, Magne Fagerhöl, who told of the findings in Malmö and of the perplexing complexities of the variants of α₁-antitrypsin as revealed by the Pi system. He suggested that the technology and structural approach taken with the abnormalities of haemoglobin could also be used to determine the molecular basis of the Pi variants. The idea had an immediate attraction to me, as I was just about to return to a medical post in New Zealand where the population was predominantly of British descent and hence where there were relatively few instances of haemoglobinopathies. Moreover, this population predictably had the typical Northern European occurrence of the variants of α₁-antitrypsin, with 4% being MZ heterozygotes and 7% MS heterozygotes (46). So, having set up the technology for protein isolation and peptide mapping in Christchurch New Zealand, the search commenced to identify the molecular basis of this variation.

Initial studies focused on the arcane multiple banding of the Z protein, in the belief that the blocking of the intracellular processing of the Z protein resulted from aberrant glycosylation. It soon became apparent however, that the underlying abnormalities of the antitrypsin variants, like those of the abnormal haemoglobins, were due to single amino acid substitutions. During a visit to Laurell in 1972, I told him of our plans to analyse the S variant of α₁-antitrypsin by mapping its tryptic peptides. Malmö, by this time, had in place a system for the large-scale isolation of the Z variant, which they planned to similarly peptide-map. By 1975, when Laurell visited New Zealand, the peptide mapping of the S variant had been completed (47) and we were able to show him that its abnormality was due to the substitution of a glutamic acid by a



α_1 -Antitrypsin and Carl-Bertil Laurell

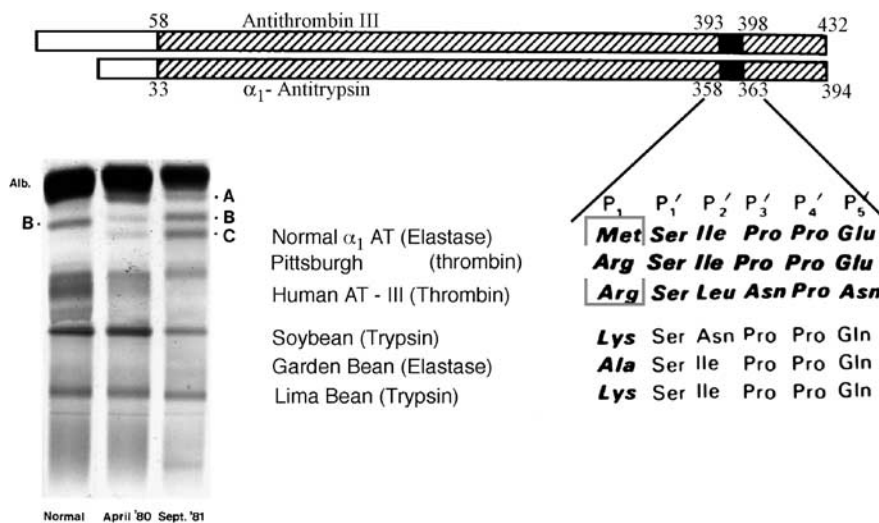


Figure 3. Antitrypsin Pittsburgh. Alignment of the sequences (above), and comparison with the known reactive centers of the smaller protease inhibitors (below) revealed the homologous reactive centers of antithrombin and α_1 -antitrypsin. This deductive siting was confirmed by the identification by Owen (62) of the mutation in the Pittsburgh variant of α_1 -antitrypsin, where the mutation of the reactive center methionine to arginine converted the antitrypsin to an inhibitor of thrombin. The use of agarose gel introduced by Laurell (16) greatly increased the discrimination of plasma protein electrophoresis, as illustrated on left with the separation of the Pittsburgh (C) from normal (B) α_1 -antitrypsin. Note also the presence of proalbumin (A) and the variation in all these components in quiescent (April 1980) versus the acute phase (September 1981) states (reproduced with permission from Ref. (62) Copyright Massachusetts Medical Society). (View this art in color at www.dekker.com.)

valine. Soon after this the abnormality of Z antitrypsin was solved by Jeppsson (48), coincidental with the independent success of Yoshida and colleagues (49) in the US. Both groups identified the replacement of a glutamic acid, different from that in the S variant, by a lysine.

THE REACTIVE SITE AND THE PITTSBURGH VARIANT

An unexpected bonus from the identification of the S and Z variants came with the sequencing of the peptides adjacent to the mutations (50). As we now know, the S mutation affects the glutamate at residue 264 and the Z mutation the glutamate at 342. So further sequencing, by Owen and Brennan from the Christchurch group, soon bridged the two mutations to give a substantial fragment of the overall sequence of α_1 -antitrypsin (51-53). The bonus was the finding that this extended sequence also contained a peptide fragment (54) that had previously been recognised by James Travis from Athens Ga, as the putative reactive centre of the molecule (40,55). This intuitive deduction by Travis was based on the close homology of the sequence of this fragment with that of the reactive centres in the smaller and totally unrelated protease

inhibitors of beans and other plants (Figure 3). But at this stage a controversy arose. The large polypeptide sequence from α_1 -antitrypsin, determined in New Zealand (56) and independently in Japan (57), was shown by Boswell (58) to be homologous to that of the carboxy-terminus of another plasma protease inhibitor, antithrombin. Moreover, the alignment of the two sequences clearly identified homologous reactive centres in both molecules. The controversial conclusion from this, that the reactive centre of antitrypsin was near the carboxy-terminus of the molecule, was directly contrary to the findings of numbers of other groups, principally in the USA, who sited it at the amino-terminus. The reason for this geographical polarisation of results later became clear. The US groups could afford the newly introduced automated peptide-sequenators, as opposed to the tedious manual sequencing techniques used elsewhere. But the sequenators brought with them an unrecognised trap, as their preparative procedures can result in the cyclisation and hence blockage of amino-terminal glutamates as present in α_1 -antitrypsin. As a consequence only a single sequence appeared following cleavage of the active centre of antitrypsin, implying an amino-terminal placement of the active site. The sequenator results, all from first-class laboratories, were so consistent in supporting this apparent amino-terminal placement that the controversy



persisted (59,60) well after other results unambiguously showed the opposite to be true. The way the debate was finally settled provides a clear example of how a clinical finding can break through scientific deadlocks to open unexpected new understandings.

Dr Jessica Lewis, a haematologist in Pittsburgh Pa, reported in 1978 a case of a severe bleeding disorder, due to a variant antitrypsin that acted as an inhibitor of the thrombin-fibrinogen interaction (61). Soon after this, the recognition of the homology and nature of the reactive sites of antitrypsin and antithrombin suggested a likely explanation, that was confirmed (62) by the analysis of the variant Pittsburgh antitrypsin (Figure 3). The peptide map of the Pittsburgh variant showed the replacement of the reactive centre methionine by an arginine, resulting in a change of function of the antitrypsin, from being an inhibitor of elastase to that of a highly active inhibitor of coagulation proteases. The finding unequivocally confirmed the carboxy-terminal position of the active site and showed how the specificity of this family of inhibitors was critically dependent on the amino acid at its reactive centre. This natural experiment in protein engineering provided the first example of the way in which a single mutation can completely change the function of a protein. Furthermore, the independence of this new inhibitor of coagulation from heparin control also settled another debate, in thrombosis research, by showing that heparin acts directly on antithrombin by activating an inherent inhibitory activity. Yet another finding from this remarkable case opened a new field in biochemistry. The investigation by Brennan (63,64) of the unexpected presence of proalbumin in the plasma of the Pittsburgh patient led to the first (and still largely unacknowledged) recognition of the nature and identity of the propeptide-cleaving enzyme that has a key function in metabolism and endocrinology.

STRUCTURE AND DEFECT IN PROCESSING

By 1978 a close collaboration had been established between the Malmö and Christchurch groups with the aim of characterising the full sequence and the nature of the heterogeneity of α_1 -antitrypsin. The protein sequence was completed in 1982, to give definitive placements of the reactive site, the S and Z mutations and the points of attachment of the three oligosaccharide sidechains of the molecule (65). The multiple electrophoretic banding of α_1 -antitrypsin was shown to be primarily due to the presence of different glycoforms of the protein, each with variations in the

antennary structure (66) of their oligosaccharides (67). Further contributions to the heterogeneity due to polymorphisms and post-translational modifications were apparent on comparison of the protein and cDNA sequences of α_1 -antitrypsin (68). The Sweden:New Zealand collaboration continued further, to establish the cause of the deficiency of the Z variant. Bathurst, Stenflo and others (69–71) using mRNA isolated from normal (MM) and variant (ZZ) livers, showed both messages had equivalent rates of translation and of subsequent glycosylation in cell-free systems. Injection of the mRNAs into a surrogate cell, the toad oöcyte, by Foreman and by Errington (72,73), confirmed that there was equivalent initial synthesis of the two proteins. But whereas all of the M protein was secreted, most of the Z protein was blocked within the oöcyte at the final stage of processing. The conclusion by 1986 from these studies was that the mutation in Z antitrypsin probably “caused a minor perturbation of structure that affects the solubility of the incompletely processed protein (74,75).”

It was clear that any further understanding of the molecular pathology of the alpha-1 syndrome required a detailed knowledge of the three-dimensional structure and function of α_1 -antitrypsin. Huber and colleagues achieved the breakthrough in Munich in 1984 by solving the crystallographic structure of α_1 -antitrypsin (76), using material provided by Laurell. This first structure was initially considered a disappointment, as the antitrypsin was not in the active inhibitory form but had been cleaved at its reactive centre. In fact this unintentional cleavage provided a stimulus to research in the field far beyond that expected from just the structure itself. The cleaved form, with its change from a five-stranded to a six-stranded A-sheet (Figure 4a) provided an enigma and a challenge to structural biology and biochemistry as a whole. No protein had been known to undergo such a remarkable change in its folding. Subsequent completion of the structure of intact α_1 -antitrypsin (77) confirmed that cleavage of the reactive centre results in its displacement to the other end of the molecule, through a distance (huge in molecular terms) of 70Å. The reason for this extraordinary conformational change became apparent with the solving of the structure of the antitrypsin-trypsin inhibitory complex by Huntington in 2000 (78) (Figure 4b). The solving of this structure completed what is now a video sequence of structures showing how this unique mobile mechanism gives the serpin family of protease inhibitors the selective advantage that makes them the predominant inhibitors in higher organisms. The forced displacement drastically distorts the structure of the protease and results in its irreversible inactivation. Thus α_1 -antitrypsin is a suicidal



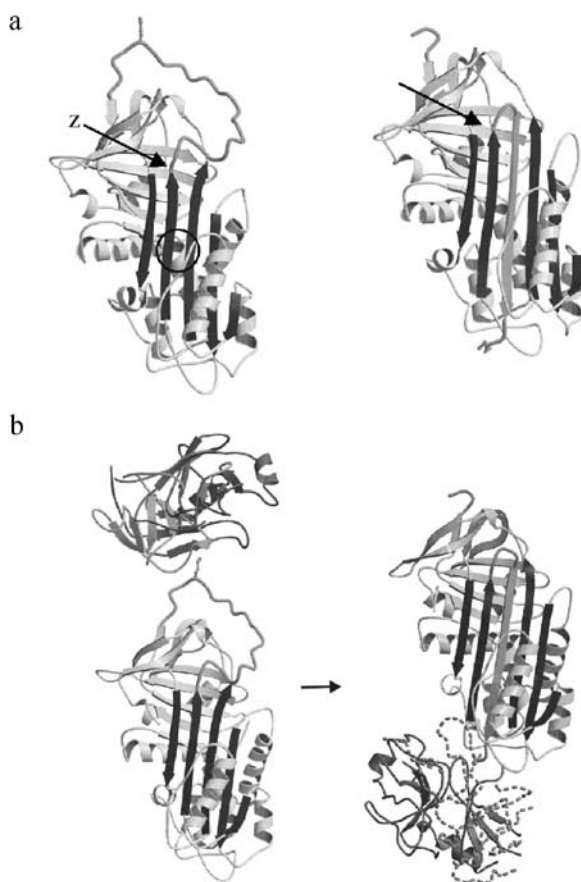


Figure 4. Structure of α_1 -antitrypsin. (a) The first crystallographic structure of α_1 -antitrypsin on the right (76) showed how the cleaved reactive center loop (yellow) is incorporated as a middle strand in the main β -sheet of the molecule (red). The intact molecule on the left shows the exposed centre. The vulnerable hinge of the loop, where the Z mutation occurs, is arrowed, and the equally vulnerable sliding shutter region is shown circled. (b) The reason for this extraordinary change in conformation is apparent from the structure of the complex of trypsin and α_1 -antitrypsin. The cleaved serpin is seen to violently displace the protease (in purple) causing its loss of structure and consequent inactivation (78). (View this art in color at www.dekker.com.)

protein that protects the elastic tissue of the lung by efficiently and irreversibly inhibiting any elastases or other serine proteases, which diffuse away from the immediate periphery of inflammatory leukocytes.

THE SERPINS

The birth of the serpins came from the initial alignment of the carboxy-terminal sequence of α_1 -antitrypsin (79) with that of antithrombin (80)

(Figure 2). But it was the recognition of a third member, the egg white protein ovalbumin that established the homologies as a new family of serine protease inhibitors (81). This family, which we now know as the serpins, was initially called the α_1 -antitrypsin-antithrombin III-ovalbumin superfamily of serine proteinase inhibitors. The renaming (4) with the acronym, the *serpins*, was not only a matter of convenience but was made with the deliberate intention of encouraging the study of the family as a whole. This reflected my much earlier experience with haemoglobin and its abnormalities. It is generally believed that the pioneer studies on the molecular function and pathology of haemoglobin were based on Perutz's crystal structure of haemoglobin; but this assumption is only partly correct. In reality, much of the early work, and almost all the studies of the molecular pathology of haemoglobin, were based on Kendrew's higher resolution structure of myoglobin. But the real insights in these early studies came from the alignment of the two globins on the basis of their shared three-dimensional structure (82), which yielded information well beyond that obtained from the study of the individual proteins. Thus the serpins were named on the precept of the globins, with the expectation of the dividends in knowledge that would come, as with the globins, from their homologous alignments. These expectations of twenty years ago have been richly rewarded. Based on α_1 -antitrypsin as the archetype of the family, we now know that the serpins, diverse though they are in their functions, all share the same overall structure and have the same molecular mechanism of action (5,8,83–86). Even more significantly they share the same molecular pathology (87). The same mutations that in α_1 -antitrypsin predispose to cirrhosis, are found in antithrombin to result in thrombosis, in C1-inhibitor to result in angioedema and in neuroserpin in dementia (7,11). A notable example is the common Z variant of α_1 -antitrypsin due to the mutation of a glutamate to a lysine, at the hinge-point of the reactive site loop (Figure 4a,b). The pathological significance of this particular mutation has been highlighted by its independent occurrence in two other serpins: in heparin cofactor II in man (88), where it results in a loss of thrombin inhibitory activity, and in the fruit-fly *Drosophila* where it causes a necrotic syndrome (12).

POLYMERISATION AND DEFICIENCY

The study of the serpins as a family soon highlighted their conformational flexibility (8,89). This was evident not only from the folding rearrangements in the first structure of α_1 -antitrypsin (Figure 4a) but



also from the identification of mutations in antithrombin and C1-inhibitor that resulted in a loss of inhibitory activity (87). These dysfunctional mutations almost all occurred in the mobile hinges of the molecule. Of special interest, was a group of mutations affecting the region critical to the shutter-like opening and closure of the main β -pleated sheet of the molecule (Figure 4b). Mutations in this shutter region (90,91) resulted in a plasma deficiency and liver aggregation of α_1 -antitrypsin, identical to that associated with the common Z variant. These changes differ from those of most genetic deficiency diseases, which usually result from a complete deficiency of an underlying protein, due to a failure of its synthesis or initial folding. As opposed to this, the Z and shutter mutations in α_1 -antitrypsin result in only a partial deficiency, with 15% of the Z protein being secreted into the plasma. With such conformationally unstable variants however, the bulk of the newly synthesised antitrypsin aggregates in the endoplasmic reticulum of the hepatocyte, predisposing to eventual cirrhosis. It is this added-on, or gain-of-function disadvantage, as opposed to a simple deficiency, that typifies what we now know as the conformational diseases.

By 1990 then, there were several outstanding questions. What is the cause of the partial deficiency? Why does the antitrypsin aggregate at this final stage of intracellular processing? What is the unique links between mutations affecting the mobile regions of the molecule and the subsequent development of cirrhosis? The first answers to these questions came from an unexpected source - the white of an egg. It was the completion by Stein of the crystal structure of the egg-white serpin, ovalbumin, which revealed the hinge and sliding movements involved in the conformational change that typifies the family (92–94). With this understanding and from accompanying studies of antithrombin, it became apparent that serpins could undergo spontaneous and pathological changes in their conformation—but what was the precise change that caused the intracellular accumulation of α_1 -antitrypsin? The solving, or more descriptively the untangling, of this problem came from the work of a young trainee respiratory physician, David Lomas, who joined the Cambridge group as a research fellow in 1990. He showed (95) that the Z antitrypsin readily formed polymers, due to the insertion of the reactive loop of one molecule into the prematurely opened β -sheet of the next (Figure 5). Electron microscopy not only demonstrated the formation of necklace-like polymers when Z α_1 -antitrypsin was incubated *in vitro*, but also confirmed that inclusions extracted from the liver were formed of entanglements of these polymers.

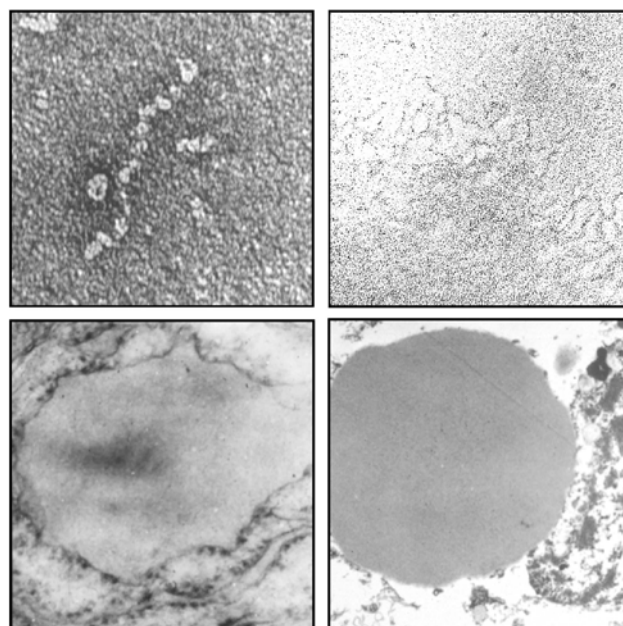


Figure 5. Polymerisation and inclusion bodies. Upper: Z antitrypsin readily undergoes loop-sheet linkage (95) to give bead-like polymers (left) which become entangled (right) to form intracellular inclusions (electron micrographs $\times 220\,000$). Below ($\times 20\,000$). Inclusions formed of entangled polymers of conformationally unstable serpins are seen with antitrypsin in the rough endoplasmic of reticulum of hepatocytes (on left), and with homologous mutations in neuroserpin in neurons, on right (10).

Strong supporting evidence for the central role of polymerisation came from an antitrypsin variant found in Japan (91) that was also associated with inclusion body formation. This Siiyama variant had a replacement of a critical amino acid (Ser 53) in the shutter region of the molecule. Isolation of the Siiyama antitrypsin from the plasma confirmed the prediction that it would readily, and in fact spontaneously, undergo polymerisation (96). Foreman, using the toad oöcyte as a surrogate for the hepatocyte, elegantly confirmed the relationship between the molecular instability and consequent partial aggregation of the variant antitrypsins (97). The conclusion that the molecular pathology of Z α_1 -antitrypsin was a direct consequence of its polymerisation was in keeping with the earlier observation of Cox (98) of the spontaneous aggregation of plasma Z antitrypsin. However, the findings of Sifers (99) suggested that the aggregation was primarily due to an impairment of intracellular degradation of the Z protein, with Perlmutter's results (100) indicating an additional genetic contribution to



this impaired turnover. So the central contribution of polymerisation to the disease syndrome remained a matter of debate until it was finally settled by a totally unexpected and landmark clinical case. And this arose because a general practitioner in upstate New York insisted on an autopsy on a patient with an atypical Alzheimer's disease.

CONFORMATIONAL DISEASE AND DEMENTIA

The misfolding (99,101) and polymerisation (95) of Z antitrypsin explained the deficiency of secretion into the plasma and hence the failure to effectively protect the lungs against proteolytic attack. But it was the consequences of the intracellular aggregation of the polymerised antitrypsin that opened wider concepts and led to the proposal of the new entity of the conformational diseases (9). These diseases each arise when an underlying protein undergoes a change in size or conformation with resultant self-association and tissue deposition. Characteristically there is intracellular aggregation of the polymerised protein with, as in the alpha-1 syndrome, a gradual and cumulative cell loss that results in a slow onset of disease. In particular, it was apparent that the cirrhosis of the Z homozygote provided a model for the progressive neurodegeneration leading to the common dementias such as Alzheimers and Parkinson's disease, as well as the prion encephalopathies (102–104). But not surprisingly there was a reluctance to accept that α_1 -antitrypsin deficiency, which was commonly considered to be a rare genetic respiratory disorder, could act as a valid model for neurodegenerative diseases. The findings that broke through this conceptual deadlock came from the autopsy on the patient with the atypical Alzheimer-like dementia.

The autopsy showed the presence of numerous inclusion bodies in the neurones of the brain. These inclusions had a striking similarity in appearance and histological staining to the inclusions seen in the liver in Z α_1 -antitrypsin cirrhosis. The reason for this became apparent from investigations by Richard Davis and colleagues in Syracuse, who showed the inclusions were composed of a brain-specific serpin-neuroserpin (105). Furthermore, genetic analysis showed the presence in the propositus and in other affected family members, of a mutation in neuroserpin at the same site as the mutation (Ser 53) that is responsible for the highly polymerogenic Siiyama variant of α_1 -antitrypsin. Lomas and colleagues showed that this late-onset dementia shared the same detailed molecular pathology as the

antitrypsin-cirrhosis, by confirming the ready polymerisation of the mutant neuroserpin and the presence of entanglements of polymers in the neuronal inclusions (10). The central contribution of polymerisation to the molecular pathology has now been put beyond any doubt by the findings of further familial encephalopathies with neuroserpin inclusion bodies (FENIB) due to other mutations in neuroserpin (106). All of these mutations are identical to those independently known to cause liver inclusions with α_1 -antitrypsin or episodic thrombosis with antithrombin (11,107). Thus the overall findings firmly establish the conformational basis of the alpha-1 syndrome, with the central feature being the polymerisation of the conformationally unstable variants (7). The findings similarly support the deduction that the common encephalopathies and dementias arise from the conformational instability and aggregation of individual neuroproteins; with the onset and severity of the neurodegeneration being associated with the rate and magnitude of protein aggregation (11,106).

A practical bonus from the realisation of the shared molecular pathology of the serpinopathies and the conformational dementias, is the prospect of shared approaches to therapy. Pharmaceutical companies are now making a huge research effort with the incentive of finding treatments for Alzheimer's and Parkinson's disease, which is also likely have relevance for the treatment of the serpinopathies. In return the serpins, and α_1 -antitrypsin in particular, currently provide the only molecularly-defined examples of conformational diseases on which to establish the design of structure based therapies.

CONCLUSION

This has been an account, from a personal viewpoint, of how the seminal finding of Carl-Bertil Laurell in 1962 opened completely new fields of understanding in biology and medicine. The finding of the deficiency of α_1 -antitrypsin provided the handle and the motivation for research that has established α_1 -antitrypsin as an archetypal protein, not only for the serpins but also for the elucidation of the conformational diseases in general. It is appropriate that this account should be published in a respiratory journal, as the key feature of this initial handle was the association of the deficiency with the development of emphysema. A theme too, in this account, has been the contribution of clinical medicine in providing the breakthroughs that have placed α_1 -antitrypsin and the serpins at the forefront of biomedical research. A respiratory physician in Malmö decided to carry out plasma electrophoresis on all his



patients, a haematologist in Pittsburgh followed-up an unusual haemorrhagic disorder, physicians in Japan further investigated unusual liver inclusions, and a practitioner and pathologists in up-State New York pursued a case of atypical neurodegenerative disease. The bringing together of all these disparate findings is a consequence of the way Laurell provided, with α_1 -antitrypsin, an immediate focus for the field and an inspiration for future research. He backed up his initial finding with the development of new technologies, such as rocket immunoelectroassay (108) that facilitated this research. But most of all he motivated his younger colleagues by the precepts he set in the rigour and integrity of his science, together with the warmth of his personality and his enthusiasm for research.

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