Recent Advances in CLINICAL IMMUNOLOGY

EDITED BY
R. A. THOMPSON

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Preface

The impact of immunology on clinical medical practice has continued, and indeed has increased, since the appearance of the last volume in this series.

The main advances have come from a wider application of monoclonal antibodies and DNA technology to dissect the nature and function of molecules and cells. To understand the events occurring in immune reactions attention has been focused on cell membrane structure and function. This volume deals with these aspects of the new ideas in immunology.

The varied guises of the phagocytic monocyte/macrophage cell have recently been uncovered by the use of monoclonal antibodies and Dr Crocker discusses these interesting cells. A unifying concept of the nature of the various anti-proteases in plasma is excitingly portrayed by Drs Boswell and Carrell, while the nature of the interleukins, and the important receptors for activated complement on cell membranes are discussed by Drs Cannon and Dinarello, and Gordon Ross respectively.

In the field of autoimmunity, one of the more interesting recent concepts has been that of autoantibodies interfering with cell function by interacting with or blocking specific receptors which control the activity of the cell. Dr Dawkins and his colleagues widely review this interesting topic in their chapter.

One of the major factors contributing to the development of clinical immunology in recent years has been the recognition of AIDS, and the research on the causation and nature of this fearsome condition. Dr Michael Gottlieb discusses the important topic of the clinical management of AIDS patients, at present defensive and largely empirical, but hopefully, by the time this volume is published, the subject of even more stirring and effective advances.

The treatment of B cell malignancy in its various forms is largely by the newly developed cytotoxic regimes, and these are carefully reviewed by Drs Westbrook and Golomb. The applications of specific immunological intervention to clinical management are discussed in the penultimate two chapters. The first is an update of the established practice of allergen hyposensitisation or immunotherapy, the second is the late 20th century concept of the control of fertility by immunological means. The final chapter illustrates the contribution of DNA technology

As always the editor gratefully acknowledges the understanding and collaboration of the distinguished contributors to this volume. While the contents are not intended to cover the field of clinical immunology comprehensively, they highlight the growing edges of the subject, and inevitably underscore the importance of immunology to medicine, and also the importance of clinical observations to the understanding of fundamental immunology.

Birmingham, 1987 R.A.T.

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Contents

1. Clinical aspects of the serpins — a family of plasma proteinase inhibitors

D. Ross Boswell R. Carrell

The name SERPIN was recently coined (Carrell & Travis, 1985) to describe a superfamily of serine proteinase inhibitors of mammalian blood plasma. The members of this superfamily, which includes the key inhibitors of the inflammatory cascades (Table 1.1), show similarity of amino acid sequence (Carrell et al, 1979; Hunt & Dayhoff, 1980). This implies similarity of structure and function, since the three-dimensional structure and thus the function of a protein is exactly specified by its sequence. Like other superfamilies such as the globins and the cytochromes, the serpins are presumed to be derived by divergent evolution from a prototypical molecule, in this case the archeserpin.

Table 1.1 Major proteinase inhibitors of human blood plasma

Inhibitor	Concentration (μmol/l)	Mass (Daltons)
Serpins		
α_1-Antitrypsin	25	51 000
α_1-Antichymotrypsin	7	69 000
Antithrombin-III	2	61 000
C1-inhibitor	2	104 000
α_2-Antiplasmin	1	70 000
Heparin cofactor II	1	66 000
Plasminogen Activator inhibitor	0.0001	50 000
Non-serpins		
α_2-Macroglobulin	4	720 000
Inter-α-trypsin inhibitor	3	160 000

The serpin superfamily includes proteins with no known antiproteinase activity such as angiotensinogen (Doolittle, 1983; Kageyama et al, 1984), ovalbumin (Hunt & Dayhoff, 1980) and barley Z protein (Hejgaard et al, 1985); we will limit the scope of this review to the plasma inhibitors: α_1-antitrypsin (Carrell et al, 1982), α_1-antichymotrypsin (Chandra et al, 1983), antithrombin-III (Petersen et al, 1979), heparin cofactor II (Church et al, 1985), α_2-antiplasmin (Lijnen et al, 1982; Holmes et al, 1986), C1-inhibitor (Davis et al, 1985; Salvesen et al, 1985) and, briefly, plasminogen activator inhibitor (Loskutoff, 1986; Pannekock, 1986) and angiotensinogen.

STRUCTURE AND MECHANISM

The only member of the superfamily for which a three-dimensional structure has been determined is α_1-antitrypsin, and the known structure is not of the native inhibitor but of a cleaved, inactive form (Loebermann et al, 1984). In this structure the peptide bond of the reactive site has been hydrolysed, and the molecule has sprung open

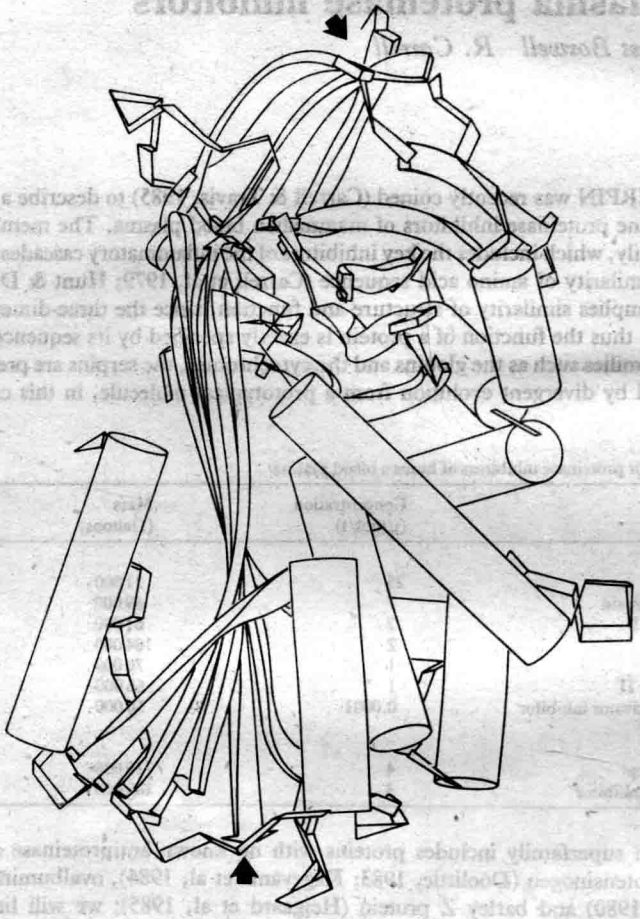

Fig. 1.1 The structure of human α_1-antitrypsin cleaved at its reactive site.
α-Helical regions are represented as cylinders, β-pleated regions as broad ribbons, and regions of indeterminate secondary structure as flat ribbon segments. The major structural feature is a large near-planar six-stranded sheet, here seen side-on. The arrows indicate the residues which in the intact inhibitor form the reactive site.
Figure prepared by Dr Arthur Lesk from coordinates supplied by Prof Robert Huber, reprinted from Boswell & Bathurst (1985) by permission of Pergamon Press.

so that the reactive site residues are at opposite poles some 70Å apart (Fig. 1.1). The exact conformation of the reactive site and of the complex formed between proteinase and inhibitor remain unknown, because the native inhibitor is by its nature metastable and is unable to form regularly diffracting crystals for X-ray structure determination.

It seems that the active inhibitor offers its target proteinase a reactive site (Table 1.2) which is on an external loop of the polypeptide chain. The loop is highly stressed by the strong tendency of the molecule to spring from its metastable native state towards the relaxed state found in the cleaved form. This stress produces strain in the reactive site peptide bond which causes it to mimic a transition state (Lienhard, 1973) in the mechanism of serine proteolysis (Huber & Bode, 1978). When the proteinase attempts to cleave the bond, the proteolytic mechanism is arrested at this state

Table 1.2 Reactive centres and targets of serpins

Inhibitor	Reactive centre sequence						Target
	P_2	P_1	P_1'	P_2'	P_3'	P_4'	
α_1-Antitrypsin	Pro	Met	Ser	Ile	Pro	Pro	Elastase
Antithrombin-III	Gly	Arg	Ser	Leu	Asn	Pro	Thrombin
Heparin cofactor II	Pro	Leu	Ser	Thr	Gln	Val	Thrombin
C1-Inhibitor	Ala	Arg	Thr	Leu	Leu	Val	C1r, C1s
α_1-Antichymotrypsin	Leu	Leu	Ser	Ala	Leu	Val	Cathepsin G
α_2-Antiplasmin	Ala	Arg	Met	Ser	Leu	Ser	Plasmin

The serpins function by offering their target proteinases a peptide bond at which hydrolysis is attempted but cannot be completed. The amino acids around this reactive site are designated P_1, P_2, . . . towards the N-terminus of the inhibitor and P_1', P_2', . . . towards the C-terminus. The serine proteinases show primary specificity for the P_1 residue, and it is by this that the serpins are broadly classified.

(Kraut, 1977). The inhibitor and its target proteinase form 1:1 complexes which are, or are capable of becoming, covalently bonded (Moroi & Yamasaki, 1974; Longas & Finlay, 1980) but which can release the proteinase by slowly dissociating in both a forward direction giving cleaved inhibitor (Loebermann et al, 1982) and a backward direction giving virgin inhibitor (Beatty et al, 1982). This dissociation is important clinically in that proteinases initially bound in plasma by serpins can be taken up by α_2-macroglobulin which has a different mechanism of inhibition (Feldman et al, 1985), and which allows entrapped proteinases to retain activity towards small substrates. In this way, 'esterase' activity, initially inhibited by the serpin, may be slowly restored both in vivo and in vitro.

The tertiary structure of the major part of the serpin molecule can be considered to act as a scaffolding which supports the reactive centre loop. Members of the Bowman-Birk family of small plant protein proteinase inhibitors (Laskowski & Kato, 1980) have a similar reactive centre and presumably a similar mechanism, but in these the strained loop is produced by disulphide bridges (Mitsui et al, 1979), and their overall molecular architecture is quite different. It seems likely that the similarity of their reactive centre sequences to those of the serpins (Fig. 1.2) represents convergent evolution.

The serpin tertiary structure, together with its carbohydrate sidechains, confers bulk and charge sufficient to prevent loss from plasma by glomerular filtration. Human α_1-antitrypsin has been synthesised in vitro without the addition of the carbohydrate sidechains (Travis et al, 1985) and the resulting naked protein has decreased in vitro stability but normal inhibitory activity. Normal human α_1-antitrypsin has a circulating half-life of 50 hours in the rabbit, but the naked α_1-antitrypsin has a half-life of only 8 hours and is taken up by the reticuloendothelial organs. It seems that the carbohydrate serves to suppress scavenging of the circulating inhibitor by the reticuloendothelial system.

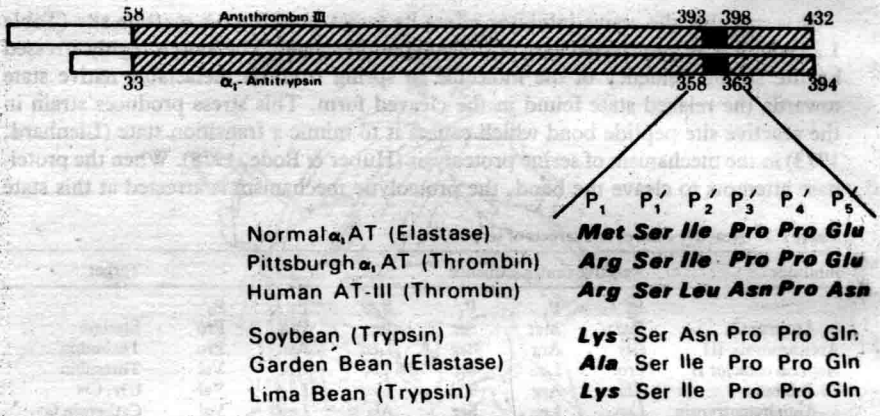

Fig.1.2 Amino acid sequences of α_1-antitrypsin with 394 residues and antithrombin-III with 432 residues.

The sequences can be aligned to give a 30% identity in structure over most of their length (hatched area). This brings in alignment the reactive centres of both molecules (solid area). These centres are compared with the known reactive centres of plant proteinase inhibitors. The P_1 residue acts as a bait that determines the specificity of enzyme inhibition as indicated. The change in this P_1 position from a methionine to an arginine in α_1-antitrypsin Pittsburgh explains the change in inhibitory specificity from elastase to thrombin.

Reprinted from Carrell et al (1983) by permission of the New England Journal of Medicine.

TARGET SPECIFICITY

The serpins select target proteinases by providing as bait a cleavage point at the P_1-P_1' reactive site (Laskowski & Kato, 1980), and they can be generally classified according to the amino acid in the P_1 position. α_1-Antitrypsin is a Metserpin and its targets would be expected to be proteinases which specifically cleave at small hydrophobic residues; antithrombin-III, α_2-antiplasmin and C1-inhibitor are Argserpins and α_1-antichymotrypsin is a Leuserpin. This classification serves to skirt the rather embarrassing situation that, because the physiological functions of the inhibitors are only now becoming apparent, the names historically applied to them are in some cases misinformative. Although α_1-antitrypsin does inhibit trypsin, it is a rather poor trypsin inhibitor. It has been argued by Travis & Salvesen (1983) that a physiologically effective inhibitor must show very rapid association with its target. The in vitro demonstration of 'progressive' (meaning slowly-progressive) inhibition of, for example, thrombin by α_1-antitrypsin is now reckoned to be unimportant in vivo. Kinetic data (Table 1.3) support the proposition that the true physiological target of α_1-antitrypsin is neutrophil elastase.

The specificity conferred by the P_1 residue is modulated by features remote from the reactive centre, and this is believed to be the basis of the targeting of the Argserpins towards different arginine-specific proteinases. There is evidence in some members of the superfamily that specialised function is particularly likely to be related to the N-terminal region of the molecule, which is on the surface and (at least in α_1-antitrypsin) does not have a rigid conformation. The activity of antithrombin-III is under particular control, in that it is a rather poor inhibitor of thrombin until it is complexed

Table 1.3 Half-times of association of Serpins with proteinases

P_1	α_1AT Met	α_1AC Leu	AT3 Arg	C1-Inhib Arg	α_2AP Arg	α_1AT mutants Pitts Arg	α_1AT mutants Valyeast Val
Human neutrophil elastase	0.0006	—	—	—	—	20	0.002
Human thrombin	800	—	0.5	—	—	0.1	—
Human kallikrein	9000	—	1000	30	—	0.4	—
Human Xa	200	—	200	—	—	2	—
Human XIa	600	—	2000	3000	—	0.08	—
Human XIIf	—	—	7000	200	—	2	—
Human plasmin	200	—	—	—	0.03	0.2	—
Human cathepsin G	0.1	0.005	—	—	—	2	60

Times in seconds, calculated from second-order association rate constants at 23°C and normal plasma concentrations, rounded to one significant figure. Unpublished results authors' laboratory, and from Scott et al (1985), and Travis & Salvesen (1984).

with heparin (Rosenberg & Damus, 1973). The molecular mechanism for this activation is not known; it is likely that the binding of heparin to antithrombin-III does not alter its inherent reactivity but reveals the reactive centre by a conformational change, making it accessible to the target proteinase. The acidic heparin appears to bind to basic residues on the surface of antithrombin-III, particularly in the N-terminal region. The binding of α_2-antiplasmin to fibrin by Factor XIII is a function of the N-terminal region (Kimura et al, 1985) although in this case it is reinforced by a similar binding site in the unique basic C-terminal prolongation of α_2-antiplasmin. We see further evidence of specialisation of the N-terminal region in angiotensinogen, since it is from here that renin cleaves the 10-residue peptide angiotensin-I (Wintroub et al, 1984).

SYNTHESIS AND CONTROL

Although there has been demonstration of α_1-antitrypsin synthesis by macrophages (Isaacson et al, 1981) and of antithrombin-III synthesis by endothelial cells (Chan & Chan, 1979), it seems that the liver is the major plasma site of synthesis of all the serpins which circulate in plasma. A clear-cut illustration of this is the conversion of the recipient's plasma to donor α_1-antitrypsin phenotype that occurs following liver transplantation (Putnam et al, 1979). The rate of synthesis of many of the serpins varies with the physiological state of the subject: acute infection, inflammation and trauma induce an acute phase plasma protein reaction (Schreiber et al, 1982; Laurell, 1985) thought to be mediated by interleukin-1 (Dinarello, 1984) in which the rates of synthesis and release of α_1-antitrypsin and α_1-antichymotrypsin increase up to five-fold (Ganrot, 1974). Antithrombin-III is a negative acute phase reactant; its rate of synthesis and release decreases in an acute phase reaction (Owens & Miller, 1980). The acute phase responses of heparin cofactor II, α_2-antiplasmin, C1-inhibitor and angiotensinogen have not been described. The factors controlling plasma concentration other than in the acute phase reaction have not been determined: there is no evidence for feedback control mechanisms. High oestrogen states, such as pregnancy, and oestrogen therapy boost α_1-antitrypsin and α_1-antichymotrypsin plasma concentrations (Ganrot, 1972).

The plasma serpins are all glycosylated and show microheterogeneity depending in part on the relative proportions of triantennary and biantennary carbohydrate side-chains (Vaughan & Carrell, 1981). It has been shown for α_1-antitrypsin that the pattern of glycosylation varies with factors such as acute phase reaction and oestrogen effect (Vaughan et al, 1982), but the significance of these changes is not known. A further contribution to the microheterogeneity of α_1-antitrypsin is made by cleavage of an N-terminal peptide (Jeppsson et al, 1985).

CLINICAL SYNDROMES OF CONGENITAL DEFICIENCY

α_1-Antitrypsin

The association of pulmonary emphysema with α_1-antitrypsin deficiency was recognised more than 20 years ago (Laurell & Eriksson, 1963) and is now well established as an example in which behavioural factors (cigarette smoking) and environmental factors (air pollution) interact with a genetic predisposition to disease; for reviews see Carrell et al (1982), Boswell & Bathurst (1985), Janoff (1985). Individuals with severe genetic deficiency of α_1-antitrypsin are at risk of emphysema because their lungs lack defence against the proteinases released by activated neutrophils. Those who do not smoke cigarettes develop emphysema at a greater rate than normal non-smokers, but may remain free from respiratory symptoms into their 7th decade. Those who smoke develop severe emphysema in early middle age, and have a life-span shortened by some 20 years (Janus et al, 1985). The molecular basis of the common Z and S deficiencies has been determined. In the Z-gene a G→A mutation (Kidd et al, 1983) gives a substitution [342 Glu→ Lys] (Jeppsson, 1976) which induces destruction of the incompletely processed glycoprotein within the endoplasmic reticulum so that only a small proportion of it is released from the cells (Bathurst et al, 1985); this gives a plasma concentration about 15% that of the normal M gene (Laurell & Jeppsson, 1975). In the S-gene an A→T mutation gives a substitution [264 Glu → Val] (Owen & Carrell, 1976) and introduces a false splice-site (Schindler, 1983) which is thought to lead to wastage of the mRNA. There is also a demonstrable, but minor, decrease in the in vitro stability of the S-protein (Lieberman, 1973) which gives it a slightly shortened plasma half-life. Overall, the S-gene product is present in plasma at about 60% of the concentration of the normal M-gene product (Laurell & Jeppsson, 1975). The group at risk of disease is believed to be those in whom the plasma concentration is less than 40% of normal, primarily ZZ and SZ deficiency which have frequencies of 0.04% and 0.06% respectively in the Northern European population. There are at least 40 other variants of α_1-antitrypsin (Jeppsson & Franzen, 1982), most of which are rare. Most notable is the null [-] variant, in which α_1-antitrypsin is essentially absent from plasma (Talamo et al, 1973). As expected, Pi--homozygotes and PiZ- heterozygotes suffer severe early-onset emphysema. The most common polymorphisms are M_1, M_2, M_3 and F, and are not associated with disease. The M_3 variation [377 Glu→ Asp] has been identified (J-O Jeppsson et al, personal communication), as has another variation [213 Val→ Ala] (Carrell et al, 1982; S Woo and R N Siefers, personal communication) which is perhaps M_2. Another mutation of interest is that of the P_5' residue of the reactive centre [363 Glu→ Lys], again unaccompanied by any obvious functional change (S. O. Brennan, personal communication).

The association of liver disease with α_1-antitrypsin deficiency is less clear-cut. While all those with PiZ deficiency show liver-biopsy evidence of accumulation of the abnormal protein within hepatocytes (Lieberman et al, 1972), the clinical picture can vary from devastating infantile cirrhosis (Sharp et al, 1965) to complete absence of clinical signs of liver disease. Overall, about one-sixth of PiZ deficient individuals die from liver disease (Larsson, 1978). There is also evidence that heterozygous (PiMZ) individuals may be at excess risk of liver disease (Hodges et al, 1981), although careful studies have failed to show excess risk of lung disease in this group (deHamel & Carrell, 1981). It is possible that the mechanism of the liver disease is related, as the emphysema is, to lack of proteinase inhibition. However, the weight of evidence suggests that the damage is a consequence of the derangement of hepatocyte function that results from the catabolic stress imposed by blockage in secretion of the Z-type α_1-antitrypsin. The most effective treatment of severe α_1-antitrypsin deficiency (Editorial, 1985) is to protect the lungs from damage by avoidance of cigarette smoking. Replacement of α_1-antitrypsin by infusion of a plasma protein concentrate is at present on trial, but seems unwarranted in the light of the relatively benign course of the disease in non-smokers. It may be justifiable in those patients with severe deficiency who have early emphysematous changes. There is no recognised treatment for the liver disease of severe α_1-antitrypsin deficiency except, when indicated, liver transplantation (Putnam et al, 1977).

There have been postulated associations of α_1-antitrypsin deficiency with many other conditions, but we will confine comment to paraproteinaemia, rheumatoid arthritis and twinning. The reported association with paraproteinaemia (Ananthakrishnan et al, 1979) has not been confirmed by other groups, and may possibly be an artefact. α_1-Antitrypsin has a single cysteine and thus a free thiol group which can bind by disulphide bridging to other proteins with free thiols. This mechanism may have some importance in vivo in allowing association of α_1-antitrypsin with immunoglobulins; in particular, IgA-α_1-antitrypsin complexes are found in synovial fluid from joints affected by rheumatoid arthritis (Stanworth, 1985). The recommended method for phenotyping by electrofocusing (Jeppsson & Franzen, 1982) involves reduction with a thiol reagent to remove artefacts due to disulphide complexes.

Heterozygotes for PiZ were found with about twice the expected frequency in a group of patients with severely destructive joint disease in rheumatoid arthritis (Cox & Huber, 1980). It is postulated not that the deficiency of α_1-antitrypsin was a cause of the arthritis, but that it might influence the severity of its expression.

Lieberman et al (1979) found PiS and PiZ heterozygotes with five times the expected prevalence amongst twins and their parents. To account for the observation that dizygotic and monozygotic twins were equally affected, two different mechanisms by which antiproteinase deficiency might affect twinning were postulated. One involved easier penetration of spermatozoa, the other involved lack of protection of the intercellular cement in the blastomere.

Antithrombin-III

A syndrome of recurrent thrombosis was recognised by Egeberg (1965) to be related to deficiency of antithrombin-III. Since this first description, there have been many reports of quantitative and a few of qualitative deficiency of antithrombin-III; for reviews see Bick (1982) and Mammen (1983a). Sufferers are heterozygous for the

deficiency, which is therefore transmitted as an autosomal codominant. No case of homozygous deficiency has been recognised. The population frequency of antithrombin-III deficiency is estimated at 1:2000 to 1:10000, but among those patients who have had repeated thrombotic illnesses before 40 years of age, the frequency may be as high as 1:40. The gene defects are heterogeneous, and as in other genetic diseases deficiency results from failure of synthesis and from production of dysfunctional molecules. Quantitative deficiency has been shown to result from gene deletion in some cases, but in others the gene is grossly intact (Prochownik et al, 1983). Three examples of qualitative deficiency with identified abnormalities have been described; in each case a key functional amino acid has been replaced. In antithrombin Toyama [47 Arg→ Cys] (Koide et al, 1984) and antithrombin Rouen [47 Arg→ His] (Carrell et al, 1986) there is loss of heparin binding and thus a failure of activation. In antithrombin Denver [394 Ser→ Leu] (Stephens et al, 1985) there is a mutation of the P_1' residue of the reactive centre resulting in loss of inhibitory activity. Antithrombin Chicago (Bauer et al, 1983) has increased heparin affinity with impaired activation; the amino acid substitution is not yet known.

Long-term treatment with coumarin anticoagulants is effective in increasing antithrombin-III activity and controlling the thrombotic tendency: it seems that the mechanism for this effect may be reduction of the normal catabolism of antithrombin-III by background activation of the coagulation system (see C1-inhibitor below).

Heparin cofactor II

Deficiency of this newly recognised serpin, an inhibitor of thrombin activated by dermatan sulphate as well as heparin, results in thrombotic disease. The first reports of familial deficiency have recently appeared (Tran et al, 1985; Sie et al, 1985). As with antithrombin-III, loss of half the normal inhibitory capacity is sufficient to cause thromboembolic problems, and the deficiency syndrome therefore shows codominant inheritance.

C1-Inhibitor

Congenital deficiency of C1-inhibitor is associated with hereditary angioedema, first described almost a century ago (Osler, 1888); for review see Frank et al (1976). Sufferers are heterozygous for a deficiency of C1-inhibitor which is classically quantitative, although a 'variant' form with a circulating qualitatively defective C1-inhibitor is recognised. The finding that C1-inhibitor activity is reduced to 15–20% of normal rather than the 50% expected in these heterozygotes is explained by catabolism of the active inhibitor at a constant rate, independent of inhibitor concentration, by background activation of the complement system (Lachmann & Rosen, 1984). Prophylactic treatment is by boosting synthesis of C1-inhibitor with sex-hormone-derived drugs such as danazol (Gelfand et al, 1976). Acute episodes may be treated with epsilon-aminocaproic acid or, more effectively, with replacement of C1-inhibitor by infusion of a plasma protein fraction (Gadek et al, 1980).

α_1-Antichymotrypsin

There have been two recent reports (Lorier et al, 1985; Eriksson, 1985) of familial α_1-antichymotrypsin deficiency in which plasma concentrations are about 60% of normal. In some Swedish cases there was associated emphysema and liver disease,

although these may have been incidental to the deficiency. This deficiency shows autosomal codominant inheritance, with a population frequency of about 1:200 in Sweden.

α_2-Antiplasmin

Deficiency of α_2-antiplasmin has been shown to be the cause of a haemorrhagic syndrome in a few families since it was first recognised by Aoki et al (1979); for review see Mammen (1983b). Homozygotes for the deficiency suffer haemarthrosis and prolonged haemorrhage following minor trauma. In one family (Miles et al, 1982) two of five heterozygotes had histories suggestive of mild haemorrhagic tendency with prolonged bleeding after tooth extraction and easy bruising, but in general heterozygotes are asymptomatic. Treatment is by prophylactic administration of epsilon-aminocaproic acid.

Plasminogen activator inhibitor

This newly recognised member of the serpin superfamily is a tissue rather than plasma protein, being particularly associated with endothelial cells. Although it is present in plasma where it functions as an antifibrinolytic agent, its plasma concentration is so low that it is readily overwhelmed by endogenous or exogenous plasminogen activators. Its clinical significance has not yet been studied, and congenital deficiency of it has not been recognised.

Angiotensinogen

Congenital deficiency of angiotensinogen has not been recognised.

CLINICAL SYNDROMES OF ALTERED SPECIFICITY

The only proven case of this type so far described is that of α_1-antitrypsin Pittsburgh (Owen et al, 1983). Mutation of the P_1 residue of α_1-antitrypsin from Met to Arg resulted in a congenital haemorrhagic syndrome. The abnormal α_1-antitrypsin had poor anti-elastase activity but was a very good inhibitor of thrombin both ex vivo (Lewis et al, 1978) and in vitro (Owen et al, 1985). While mimicking the coagulation-inhibiting behaviour of antithrombin-III, it retained the property of α_1-antitrypsin that it had full activity irrespective of the presence of heparin. It also showed good inhibitory activity against other arginine-specific proteinases (see Table 1.3). The patient suffered repeated episodes of intractable haemorrhage, particularly when illness or trauma boosted the plasma concentration of α_1-antitrypsin in an acute phase reaction, and died from haemorrhage at the age of 14. The molecular abnormality not only explained the clinical findings, but also provided clear confirmation of the homology of α_1-antitrypsin and antithrombin-III and gave positive identification of their reactive centres (Fig. 1.2). The demonstration that a single amino acid substitution can produce a complete change in specificity has opened up the prospect of the production of targeted inhibitors by genetic engineering.

ACQUIRED DEFICIENCY OF PROTEINASE INHIBITORS

α_1-Antitrypsin and the neutrophil

When the reactive centre sequence of α_1-antitrypsin was first determined, it seemed