Chymotrypsin inhibitory activity of normal C1-inhibitor and a P1 Arg to His mutant: Evidence for the presence of overlapping reactive centers

K.S. AULAK,¹ A.E. DAVIS III,¹ V.H. DONALDSON,² AND R.A. HARRISON³

¹ Division of Nephrology, Children's Hospital Research Foundation, and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229-2899

² Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229-2899

³ Molecular Immunopathology Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, England

(RECEIVED October 20, 1992; REVISED MANUSCRIPT RECEIVED January 12, 1993)

Abstract

C1-inhibitor is a serine proteinase inhibitor that is active against C1s, C1r, kallikrein, and factor XII. Recently, it has been shown that it also has inhibitory activity against chymotrypsin. We have investigated this activity of normal human C1-inhibitor, normal rabbit C1-inhibitor, and P1 Arg to His mutant human C1-inhibitors and find that all are able to inhibit chymotrypsin and form stable sodium dodecyl sulfate-resistant complexes. The K_{ass} values show that the P1 His mutant is a slightly better inhibitor of chymotrypsin than normal human C1-inhibitor (3.4×10^4 compared with 7.3×10^3). The carboxy-terminal peptide of normal human C1-inhibitor, derived from the dissociated protease-inhibitor complex, shows cleavage between the P2 and P1 residues. Therefore, as with α 2-antiplasmin, C1-inhibitor possesses two overlapping P1 residues, one for chymotrypsin and the other for Arg-specific proteinases. In contrast, with the P1 His mutant, the peptide generated from the dissociation of its complex with chymotrypsin demonstrated cleavage between the P1 and P'1 residues. Therefore, unlike α 2-antiplasmin, chymotrypsin utilizes the P2 residue as its reactive site in normal C1-inhibitor but utilizes the P1 residue as its reactive site in the P1 His mutant protein. This suggests that the reactive center loop allows a degree of induced fit and therefore must be relatively flexible.

Keywords: C1-inhibitor; chymotrypsin inhibitor; overlapping reactive center; P1 Arg to His mutant

The plasma serine proteases play a central role in the multicomponent cascade systems of plasma such as complement, coagulation, and fibrinolysis. Inhibition of many proteases is provided by protein protease inhibitors; these have been grouped into a limited number of "super-families." The most abundant family in plasma is that of the serine protease inhibitors (serpins). Although not all serpins are protease inhibitors, those that appear to share a common basic mechanism, albeit with specific function-based refinements (e.g., heparin modulation of antithrombin III activity [Carrell et al., 1989] and vitronectin modulation of plasminogen activator inhibitor [Salonen et al., 1989]). This mechanism is thought to de-

pend on amino acid side chains along the reactive center "loop" that mimic the natural substrate of the enzyme, and which promote binding of the inhibitor to protease. The inhibitor then corrupts the normal proteolytic process by forming a stable protease-inhibitor (substrate) complex (Moroi & Yamasaki, 1974). Under certain conditions this intermediate can be dissociated with release of a proteolyzed inactivated inhibitor and the reactivated protease. Alternatively, denaturation of the intermediate generates a sodium dodecyl sulfate (SDS)-stable covalent complex due to the formation of an acyl-bond between the carboxyl group of the P1 residue of the inhibitor and the hydroxyl group from the protease active-site serine (Matheson et al., 1991).

C1-inhibitor is the sole plasma inhibitor of C1s and C1r (Sim et al., 1979) and, with other inhibitors, contributes to the inhibition of factor XIa, factor XIIa, kallikrein, tis-

Reprint requests to: K.S. Aulak, Division of Nephrology, Children's Hospital Research Foundation, Cincinnati, Ohio 45229-2899.

sue plasminogen activator, and plasmin (Davis, 1988). These proteases cleave target proteins immediately carboxy-terminal to a basic residue. The "reactive center" of serpins is comprised of a short sequence of residues characterized by susceptibility to proteolytic attack (Carrell et al., 1987). Whereas several of these residues may play a part in defining inhibitor specificity, a major determinant of specificity is the P1 residue (the amino acid on the amino-terminal side of the susceptible bond).

Hereditary angioedema (HAE) results from reduced plasma levels of functional C1-inhibitor. Two forms of the disease have been defined. In type II HAE both alleles express C1 inhibitor; one produces a dysfunctional protein and the other a normal functional protein (Rosen et al., 1971). Most mutations in the dysfunctional proteins (~80%) are due to replacement of the P1 Arg residue with either His or Cys. Functional characterization showed that the P1 Arg to His mutants had lost most of their inhibitory activity against C1s and plasmin (Aulak, 1989; Eldering et al., 1989).

The first suggestion that a single serpin might combine inhibitory activity against chymotrypsin in addition to C1s and C1r was made using a protein purified from bovine plasma (van Nostrand & Cunningham, 1987). This protein was believed to be distinct from C1-inhibitor. Eldering et al. (1989) demonstrated that both normal human C1-inhibitor and P1 His mutants transiently expressed in cos cells were able to inhibit chymotrypsin. We here analyze the inhibition of chymotrypsin with normal human and rabbit C1-inhibitor and with two different C1-inhibitor P1 Arg to His mutants (C1-inhibitor [Ri] and [At]).

Results

C1-inhibitor-chymotrypsin complex formation

Rabbit C1-inhibitor, human C1-inhibitor, and a P1 Arg to His mutant of human C1-inhibitor (C1-inhibitor [At]) were examined for SDS-stable complex formation with bovine chymotrypsin. Figure 1 shows SDS-polyacrylamide gel electrophoresis (PAGE) of the C1-inhibitor proteins incubated with increasing concentrations of chymotrypsin. At low chymotrypsin concentrations, normal human C1-inhibitor produced small amounts of a complex of the expected size (Fig. 1A). Unreacted C1-inhibitor (At) showed a double band; this previously has been shown to be due to the loss of carbohydrate from the molecule (Fig. 1B) (Aulak et al., 1988). This mutant also formed a high molecular weight complex with chymotrypsin. Furthermore, the complex was double banded, which indicated that both bands seen in the unreacted protein were active. A protein from rabbit plasma was purified using a C1-inhibitor-specific method and shown to be C1-inhibitor by complex formation with human C1s (data not shown) and by reactive center sequencing (Fig. 2). This

Fig. 1. Complex formation with chymotrypsin. Human C1-inhibitor (4.5 µg; **A**), C1-inhibitor (At) (4.5 µg; **B**), or rabbit C1-inhibitor (4.5 µg; **C**) was incubated with increasing amounts of α -chymotrypsin (0, 0.16, 0.31, 0.62, and 1.25 µg in lanes 1–5, respectively) for 20 min at 37 °C. The reaction was stopped with phenylmethylsulfonylfluoride and loaded onto a 7.5–20% polyacrylamide gradient gel using nonreducing loading buffer.

protein also produced a complex of appropriate molecular weight after incubation with chymotrypsin (Fig. 1C).

Kinetics of chymotrypsin inhibition

The kinetics of the inhibition reaction were measured with normal human C1-inhibitor and with the P1 Arg to His mutant, C1-inhibitor (Ri). C1-inhibitor (Ri) was used because it had no detectable normal C1-inhibitor as assessed

Normal numbering	P4	P3	P2	P1	P'1	P'2	P'3	P'4	P'5	P'6	P'7	P'8	P'9	P'10	P'11	P'12	P'13	P'14
C1-inhibitor (human)	V	A	R	T	L	L	V	F	E	V	Q	Q	P	F	L	F	V	L
C1-inhibitor (rabbit)	V	A	R	S	L	L	I	F	E	V	Q	Q	P	F	L	F	L	L

Fig. 2. Alignment of rabbit C1-inhibitor with human C1-inhibitor. Rabbit C1-inhibitor was cleaved with Ps.a.elastase, the carboxy-terminal peptide was isolated, and its amino-terminal sequence was determined (as described in Materials and methods).

by sequence analysis of *Pseudomonas aeruginosa* (Ps.a.) elastase-released reactive center peptides, quantitation of residual C1s inhibitory activity, and by sensitivity to trypsin digestion (data not shown). Any inhibitory activity detected could therefore be reliably assigned to the mutant protein.

The amount of active chymotrypsin was assessed by active-site titration using *p*-nitrophenyl acetate (Kezdy & Kaiser, 1970), and the second-order association rates for C1-inhibitor with chymotrypsin were determined using the synthetic substrate Suc-Ala-Ala-Pro-Phe *p*-nitroanilide. The association rate constant measured for normal human C1-inhibitor was 7.3×10^3 , and that for the P1 Arg to His mutant C1-inhibitor(Ri) was 3.4×10^4 .

Determination of the P1 residue

The P1 residue is a major determinant of serpin specificity. Proteases inhibited physiologically by C1-inhibitor cleave at Arg-X peptide bonds. In contrast, chymotrypsin cleaves on the carboxy-terminal side of bulky hydrophobic residues. Potempa et al. (1988) have shown that α 2-antiplasmin can utilize overlapping P1-P'1 sites, with that for plasmin being the Arg-Met and that for chymotrypsin being the Met-Ser. As the physiological P1 residue in C1-inhibitor is Arg⁴⁴⁴, which would be a highly improbable chymotryptic cleavage site, we were interested to see whether a similar phenomenon might occur with C1-inhibitor. The carboxy-terminal peptides from the dissociated complexes were isolated by reverse-phase high-performance liquid chromatography and subjected to amino-terminal sequence analysis (Fig. 3). The data indicate that the C1-inhibitor Arg to His mutant utilizes

His⁴⁴⁴ as the P1 residue, whereas normal C1-inhibitor utilizes Ala⁴⁴³.

Discussion

The anti-chymotryptic inhibitory activity of C1-inhibitor was initially assessed by its ability to form SDS-stable complexes with chymotrypsin. At a low molar ratio of chymotrypsin to normal human C1-inhibitor, a band of the predicted size for a C1-inhibitor-chymotrypsin complex was observed. A large amount of the intact 100-kDa protein also was converted to a 97-kDa form. This results from cleavage near the amino-terminus of C1-inhibitor. Schoenberger et al. (1989) showed that, even at very low ratios of chymotrypsin, chymase, or cathepsin G, C1-inhibitor was cleaved rapidly at the Phe³³-Val³⁴ peptide bond. We have also observed that cathepsin G cleaves only in the amino-terminal region of human C1-inhibitor and have shown that this is true also for the P1 Arg to His mutant, C1-inhibitor (At) (data not shown). Schoenberger et al. (1989), however, did not detect any complex formation with chymotrypsin. The reason for this is unclear. Proteolytic cleavage in the amino-terminal region of C1-inhibitor (At) and rabbit C1-inhibitor is less apparent than with normal human C1-inhibitor and appears to require higher concentrations of chymotrypsin. This may reflect the relative efficiencies of inhibition of the protease by the different C1-inhibitor preparations. Analysis of the kinetics of inhibition of normal human C1-inhibitor and C1-inhibitor (At) shows that the latter has an association rate about five times that of the former and therefore is a more effective inhibitor of chymotrypsin. The absence of

Normal numbering	P4	P3	P2	P1	P'1	P'2	P'3	P'4	P'5	P'6
Human C1-inhibitor sequence	S	V	A	R	T	L	L	V	F	E
Isolated Peptide	-	-	-	R	T	L	L	V	F	E
C1-inhibitor(At) sequence	S	V	A	H	T	L	L	V	F	E
Isolated Peptide	-	-	-	-	T	L	L	V	F	E

Fig. 3. Sequence of the inhibitor-derived carboxy-terminal peptide isolated from the dissociated chymotrypsin-C1-inhibitor complex. The sequence of the unreacted protein is shown above each peptide sequence. Arrow indicates the position of cleavage.

amino-terminal cleavage of rabbit C1-inhibitor suggests that its rate of inhibition of chymotrypsin is also greater than that of human C1-inhibitor, but no direct measurement of this has been made. At high chymotrypsin concentrations cleavage of the complexes are observed; this phenomenon also has been reported with other serpin-protease complexes (Bloom, 1977; Faulmann et al., 1987).

The P1 residue of serpins is generally regarded as the major determinant of specificity. For example, a basic P1 residue would be expected to inhibit trypsin-like proteases, and a bulky hydrophobic P1 residue would be expected to inhibit chymotrypsin-like proteases. The P1 residue of C1-inhibitor is Arg⁴⁴⁴, and from this, C1-inhibitor is predicted to be specific for trypsin-like proteases. Inhibition of chymotrypsin was therefore surprising. One clue as to a possible explanation of this activity came from the work of Potempa et al. (1988), who showed that α 2-antiplasmin can express dual specificities by using overlapping P1-P'1 sites. Trypsin and plasmin recognize Arg³⁶⁴-Met³⁶⁵ as the functional P1-P'1 site, whereas chymotrypsin recognizes Met³⁶⁵-Ser³⁶⁶. The sequence of the carboxy-terminal peptide from the dissociated C1-inhibitor-chymotrypsin complexes showed that normal human C1-inhibitor utilizes Ala⁴⁴³ (the P2 residue in physiological inhibition events) as the functional P1 residue, whereas C1-inhibitor (At) used the mutated P1, His⁴⁴⁴, as its functional P1 residue.

The overlapping P1-P'1 sites of C1-inhibitor differ from those of α 2-antiplasmin in a very fundamental way. α 2-Antiplasmin expresses two P1-P'1 sites, one with plasmin and trypsin and the other with chymotrypsin. Each protease is absolutely specific for a single site. In contrast, we have shown that for inhibition of chymotrypsin by normal human C1-inhibitor, Ala⁴⁴³-Arg⁴⁴⁴ is used as the P1-P'1 site, whereas C1-inhibitor (At) uses His⁴⁴⁴-Thr⁴⁴⁵. A single protease is therefore not restricted to attack at a single site on the molecule.

An α 1-antichymotrypsin-like sequence is present in the reactive center region of C1-inhibitor (α 1-antichymotrypsin P3-P'1, TLLS; C1-inhibitor P'1-P'4, TLLV). However, in spite of this sequence similarity, there is no evidence that C1-inhibitor utilizes the P'3-P'4 Leu-Val site in its inhibition of chymotrypsin. Thus, even though potentially high-affinity primary binding sites for chymotrypsin are located close to the normal P1-P'1 site of C1-inhibitor, the actual choice of site used in inhibition is restricted to a short segment of the reactive center loop.

Demonstration of overlapping P1-P'1 sites in serpins makes them unique among protease inhibitors. In other inhibitors, the position of the P1-P'1 residues are strictly defined. This suggests that the serpin inhibitory mechanism is different. This is further emphasized by consideration of the interactions between protease and inhibitor in the complex. While other protease inhibitor-protease complexes, such as those of Kunitz-type inhibitors, are largely stabilized by interactions between the protease and

residues within the reactive center (Bode & Huber, 1991), our analysis of the inhibition of chymotrypsin by C1-inhibitor indicates that stabilization of the initial complex must be provided by residues in positions outside the reactive center loop. These must be sufficiently strong for suboptimal specificity (Ala-Arg or His-Thr) to be imposed on the protease. In addition, because these nonreactive center interactions are likely to be sterically fixed, the overlapping dual sites for the interaction with either C1-inhibitor (At) or normal C1-inhibitor demonstrate a flexibility in the reactive center loop such that either the Ala-Arg or the His-Thr bond can be correctly aligned with the catalytic residues (especially serine 195) of the protease reactive center. This requires a flexibility of around 4 Å (possibly generated as ± 2 Å from the median state). The structure of the reactive center loop in native active inhibitory serpins is as yet unknown. Current belief is that in active inhibitor serpins there is some mobility of the reactive center loop with a degree of insertion into the A sheet. The flexibility could therefore derive from a natural continual movement of residues of the hinge region into and out of the A sheet, with "trapping" of the "best-fit" P1-P'1 site in the protease reactive center. This implies a degree of induced fit in the complex.

It is not clear why only C1-inhibitor and α 2-antiplasmin appear to possess overlapping P1-P'1 sites. It is possible this phenomenon has been overlooked in other serpins. It is interesting to note that a P1 Leu to Arg mutation in α 1-antichymotrypsin is still able to inhibit chymotrypsin (Rubin et al., 1990). It is possible that this protein also uses the P2 Leu as the new P1 site as observed with C1-inhibitor. Further work using mutagenesis with these and other serpins will determine whether this phenomenon is restricted only to these proteins.

Materials and methods

Isolation of C1-inhibitor proteins

The plasmas for C1-inhibitor isolation were stored at -70°C until required. EDTA and benzamidine were added to a final concentration of 10 mM, when the plasmas were thawed. Patient At was on Danazol therapy (100 mg/day), which was discontinued for 10 days before collection of blood. Patient Ri was not receiving any medication at the time of collection. Purified C1-inhibitor, C1-inhibitor (At), and C1-inhibitor (Ri) were prepared according to the method of Harrison (1983). Purified proteins were tested and found negative for α 1-antichymotrypsin and α 1-antitrypsin by double immunodiffusion and SDS-PAGE.

Rabbit C1-inhibitor was isolated by a modification of the method of Pilatte et al. (1989). Briefly, 50 mL rabbit plasma was brought to 10 mM in EDTA, 50 μM in soybean trypsin inhibitor, and 25 μM in *p*-nitrophenyl-*p'*-guanidino benzoate (NPGb) immediately after thawing.

The precipitate from a 22.5–45% polyethylene glycol 3350 cut was dissolved in 10 mL phosphate-buffered saline (PBS) containing 50 μ M NPGB, 10 mM EDTA (jacalin-Sepharose buffer). This was then loaded onto a jacalin-Sepharose column equilibrated with the jacalin-Sepharose buffer and then washed with jacalin-Sepharose buffer + 0.5 M NaCl (washing buffer) until the OD₂₈₀ fell to near background. Bound proteins were then eluted using washing buffer + 0.125 M melibiose. Ammonium sulfate was then added to the eluted peak to a final concentration of 0.4 M and the sample applied to a phenyl-Sepharose column equilibrated with PBS + 0.4 M ammonium sulfate. The nonadsorbed peak was collected, dialyzed against PBS, and concentrated using an Amicon system with a PM10 membrane.

SDS-PAGE analysis

SDS-PAGE was performed using the buffer and sample preparation systems described by Laemmli (1970). Proteins were prepared for electrophoresis using nonreducing sample preparation buffers and the gels stained with Coomassie brilliant blue (Sigma).

Digestion of rabbit C1-inhibitor with *Ps.a.elastase*

Ps.a.elastase digestion of purified rabbit C1-inhibitor was performed by a modification of the method described by Pemberton et al. (1989). *Ps.a.elastase* (a kind gift of Dr. Morihari, Kyoto Research Laboratories, Kyoto, Japan) was incubated with rabbit C1-inhibitor at a molar ratio of about 1:200 at 37 °C for 30 min. The reaction was stopped by the addition of EDTA to a final concentration of 5 mM.

Complex formation on SDS-PAGE

C1-inhibitor was incubated with various concentrations of bovine pancreatic α -chymotrypsin (Worthington) at 37 °C for 20 min. The reaction was stopped by addition of phenylmethylsulfonylfluoride to a final concentration of 6 mM. The samples were then mixed with SDS-PAGE nonreducing sample buffer and loaded onto a 7.5–20% polyacrylamide gel.

HPLC purification of the protein fragments

The C1-inhibitor-chymotrypsin "complexes" were prepared by incubation of C1-inhibitor and chymotrypsin at a molar ratio of 1:0.8 for 30 min at 37 °C, followed by the addition of benzamidine and EDTA (each to a final concentration of 10 mM), and 10 volumes of 5% acetonitrile, 0.1% trifluoroacetic acid (TFA). The sample was heated at 85 °C for 30 min and passed through a 0.2- μ M filter. This was loaded onto a PLRP-S 300-Å column (250 $\times$ 4.6 mm; Polymer Laboratories, Church Stretton,

Shropshire, UK), equilibrated with 5% acetonitrile–0.1% TFA. After an initial wash for 10 min with 5% acetonitrile–0.1% TFA, a linear gradient to 80% acetonitrile–0.1% TFA was developed over 40 min. A flow rate of 1 mL/min was used throughout. Isolation of the carboxy-terminal fragment of rabbit C1-inhibitor generated by *Ps.a.elastase* was achieved using a Waters delta pak C18-300-Å column (3.9 mm $\times$ 15 cm; Waters Tokyo, Japan) under the same elution conditions as above.

Peptide sequence analysis

Amino-terminal sequence analysis of the carboxy-terminal reactive center peptides was performed using an Applied Biosystems protein sequencer by Dr. L. Packman at the Protein Sequencing Facility of the Department of Biochemistry, University of Cambridge, Cambridge, UK.

Kinetic analysis

Active-site titrations of chymotrypsin were carried out as described by Kezdy and Kaiser (1970) using *p*-nitrophenyl acetate. Equimolar concentrations of protease and inhibitor were incubated at 37 °C in 20 mM Tris/HCl, 150 mM NaCl, 5 mM CaCl₂, pH 7.5. Aliquots were analyzed at timed intervals for chymotrypsin activity using the synthetic substrate Suc-Ala-Ala-Pro-Phe *p*-nitroanilide. The second-order rate constants were calculated using the formula:

$$K_{ass} = \frac{1}{\text{half-life of the interaction} \times \text{enzyme concentration used}}$$

References

- Aulak, K.S. (1989). Analysis of dysfunctional C1-inhibitor proteins isolated from individuals with type II hereditary angioedema. Ph.D. thesis, University of Cambridge, Cambridge, UK.
- Aulak, K.S., Pemberton, P.A., Rosen, F.S., Carrell, R.W., Lachmann, P.J., & Harrison, R.A. (1988). Dysfunctional C1-inhibitor (At), isolated from a type II hereditary-angio-oedema plasma, contains a P1 "reactive centre" (Arg444-His) mutation. *Biochem. J.* 253, 615–618.
- Bloom, J.W. (1977). Interaction of α_1 -antitrypsin with trypsin and chymotrypsin. *J. Biol. Chem.* 253, 547–559.
- Bode, W. & Huber, R. (1991). Ligand binding: Proteinase inhibitor interactions. *Curr. Opin. Struct. Biol.* 1, 45–52.
- Carrell, R.W., Aulak, K.S., & Owen, M.C. (1989). The molecular pathology of the serpins. *Mol. Biol. Med.* 6, 35–42.
- Carrell, R.W., Pemberton, P.A., & Boswell, D.R. (1987). The serpins: Evolution and adaptation in a family of protease inhibitors. *Cold Spring Harbor Symp. Quant. Biol.* 52, 527–535.
- Davis, A.E., III. (1988). C1 inhibitor and hereditary angioneurotic edema. *Annu. Rev. Immunol.* 6, 595–628.
- Eldering, E., Huijbregts, C.C., Lubbers, Y.T., Longstaff, C., & Hack, C.E. (1989). Recombinant C1 inhibitor P1 variants. *Complement Inflammation* 6(5), 333 [Abstr.].
- Faulmann, E.L., Young, M., & Boyle, M.D. (1987). Inactivation of the proteolytic activity of mouse nerve growth factor by human C1(activated)-inhibitor. *J. Immunol.* 138, 4336–4340.
- Harrison, R.A. (1983). Human C1 inhibitor: Improved isolation and preliminary structural characterization. *Biochemistry* 22, 5001–5007.

- Kezdy, F.J. & Kaiser, E.T. (1970). Principles of active site titration of proteolytic enzymes. *Methods Enzymol.* 19, 3–20.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Matheson, N.R., van Halbeek, H.H., & Travis, J. (1991). Evidence for a tetrahedral intermediate complex during serpin–proteinase interactions. *J. Biol. Chem.* 266, 13489–13491.
- Moroi, M. & Yamasaki, M. (1974). Mechanism of interaction of bovine trypsin with human alpha-1 antitrypsin. *Biochim. Biophys. Acta* 239, 130–141.
- Pemberton, P.A., Harrison, R.A., Lachmann, P.J., & Carrell, R.W. (1989). The structural basis for neutrophil inactivation of C1 inhibitor. *Biochem. J.* 258, 193–198.
- Pilatte, Y., Hammer, C.H., Frank, M.M., & Fries, L.F. (1989). A new simplified procedure for C1 inhibitor purification. A novel use for jacalin-agarose. *J. Immunol. Methods* 120, 37–43.
- Potempa, J., Shieh, B.H., & Travis, J. (1988). Alpha-2- antiplasmin: A serpin with two separate but overlapping reactive sites. *Science* 241, 699–700.
- Rosen, F.S., Alper, C.A., Pensky, J., Klemperer, M.R., & Donaldson, V.H. (1971). Genetically determined heterogeneity of the C1 esterase inhibitor in patients with hereditary angioneurotic edema. *J. Clin. Invest.* 50, 2143–2149.
- Rubin, H., Wang, Z.-H., Nickbarg, E.B., McLarney, S., Naidoo, N., Schoenberger, O.L., Johnson, J.L., & Cooperman, B.S. (1990). Cloning, expression, purification and biological activity of recombinant native and variant human α 1-antichymotrypsins. *J. Biol. Chem.* 266, 1199–1207.
- Salonen, E.M., Vaheri, A., Pollanen, J., Stephans, R., Andreasen, P., Mayer, M., Dano, K., Gailit, J., & Ruoslahti, E. (1989). Interaction of plasminogen activator inhibitor (PAI-1) with vitronectin. *J. Biol. Chem.* 264, 6339–6343.
- Schoenberger, O.L., Sprows, J.L., Schechter, N.M., Cooperman, B.S., & Rubin, H. (1989). Limited proteolysis of C1-inhibitor by chymotrypsin-like proteinases. *FEBS Lett.* 259, 165–167.
- Sim, R.B., Reboul, A., Arlaud, G.J., Villiers, C.L., & Colomb, M.G. (1979). Interaction of 125 I-labelled complement subcomponents C1r and C1s with protease inhibitors in plasma. *FEBS Lett.* 97, 111–115.
- van Nostrand, W.E. & Cunningham, D.D. (1987). Purification of a proteinase inhibitor from bovine serum with C1-inhibitor activity. *Biochim. Biophys. Acta* 923, 167–175.