The \(\alpha\)-Macroglobulin Bait Region

SEQUENCE DIVERSITY AND LOCALIZATION OF CLEAVAGE SITES FOR PROTEINASES IN FIVE MAMMALIAN \(\alpha\)-MACROGLOBULINS

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The amino acid sequence of a 90-residue segment of human pregnancy zone protein containing its bait region has been determined. Human \(\alpha_2\)-macroglobulin, human pregnancy zone protein, and rat \(\alpha_1\)-macroglobulin, \(\alpha_2\)-macroglobulin, and \(\alpha_1\)-inhibitor 3 variants 1 and 2 constitute a group of homologous proteins; but the sequences of their bait regions are not related, and they differ in length (32–55 residues). The \(\alpha\)-macroglobulin bait region is located equivalently with residues 666–706 in human \(\alpha_2\)-macroglobulin. In view of the extreme sequence variation of the bait regions, the evolutionary constraints for these regions are likely to differ from those of the remainder of the \(\alpha\)-macroglobulin structure. The sites of specific limited proteolysis in the bait regions of human pregnancy zone protein and rat \(\alpha_1\)-macroglobulin, \(\alpha_2\)-macroglobulin, and \(\alpha_1\)-inhibitor 3 variants 1 and 2 by a variety of proteinases differing in specificity have been determined and compared with those identified earlier in human \(\alpha_2\)-macroglobulin. The sites of cleavage generally conform to the substrate specificity of the proteinase in question, but the positions and nature of the P4-P4’ sites differ. Most cleavages occur in two relatively small segments spaced by 6–10 residues; and in each case, bait region cleavage leads to \(\alpha\)-macroglobulin-proteinase complex formation. The rate at which a given proteinase cleaves \(\alpha\)-macroglobulin bait regions is likely to show great variation. Possible structural features of the widely different bait regions and their role in the mechanism of activation are discussed.

The proteinase-binding \(\alpha\)-macroglobulins (\(\alpha\)Ms) are large glycoproteins found in the plasma of vertebrates, in the hemolymph of some invertebrates, and in bird and reptile egg white. Most \(\alpha\)Ms are tetramers assembled from pairwise disulfide-bridged 180-kDa subunits (e.g. human and rat \(\alpha\)Ms), but dimeric and monomeric \(\alpha\)Ms are also known (e.g. human PZP and rat \(\alpha_1\)I, respectively) (for recent reviews, see Travis and Salvesen (1983) and Sottrup-Jensen (1987)). The active site of an \(\alpha\)M-bound proteinase is accessible to small substrates and inhibitors. This feature and the ability of \(\alpha\)Ms to form complexes with a variety of proteinases from all classes (EC 3.4.21–24) distinguish \(\alpha\)Ms from most proteinase inhibitors.

The human \(\alpha_2\)M subunit contains an exposed stretch called the bait region (Harpe, 1973; Barrett and Starkey, 1973) located near the middle of the polypeptide chain which is uniquely sensitive to cleavage by proteinases. Complex formation with a variety of proteinases is initiated by specific cleavage in that region, and the sites of cleavage have been determined for many proteinases (Sottrup-Jensen et al., 1981a; Hall et al., 1981; Mortensen et al., 1981b; Virca et al., 1983; Sottrup-Jensen and Birkedal-Hansen, 1989). Bait region cleavage triggers conformational changes in the \(\alpha_2\)M subunits resulting in the generation of tight-fitting binding sites for proteinases in the tetrameric structure. Maximally two proteinase molecules can be bound; and conceivably, each \(\alpha_2\)M dimer contains one binding site.

The conformational changes initiated by bait region cleavage also cause activation of internal thiol esters formed from Cys-949 and Glx-952 in each subunit of \(\alpha_2\)M (Sottrup-Jensen et al., 1980, 1984b). The activated thiol esters provide \(\alpha_2\)M with a potential for covalent cross-linking of the activating proteinase through \(\epsilon\)-lysyl (proteinase)-\(\gamma\)-glutamyl (\(\alpha_2\)M) bonds and also for binding of other nucleophiles present at activation (Sottrup-Jensen et al., 1981b, 1981c; Salvesen and Barrett, 1981).

As a further result of bait region cleavage, previously concealed recognition sites for receptors on a variety of cells including fibroblasts, macrophages, and hepatocytes (Debanne et al., 1975; Van Leuven et al., 1979; Gliemann and Virca, 1983) become exposed causing rapid clearance and degradation of \(\alpha_2\)M-proteinase complexes from the circulation (Ohlsen, 1971; Imber and Pizzo, 1981). These features have been investigated in detail for human \(\alpha_2\)M and are shared by most members of the \(\alpha\)M family.

The sequence of the subunit of human \(\alpha_2\)M (1451 residues) and the disulfide bridge pattern of its 360-kDa dimeric unit are known (Sottrup-Jensen et al., 1984b; Kan et al., 1985; Jensen and Sottrup-Jensen, 1986); and recently, the sequences of the subunits of rat \(\alpha_2\)M (1445 residues), rat \(\alpha_1\)Is (1453 residues), and rat pro-\(\alpha_2\)M (1476 residues) were determined (Gehring et al., 1987; Braciak et al., 1988; Aiello et al., 1988). For human PZP, about 50% of the sequence is known (Sottrup-Jensen et al., 1984c; Sand et al., 1985).

Overall, the sequences of human \(\alpha_2\)M and PZP and of the rat proteins \(\alpha_2\)M, \(\alpha_1\)M, and \(\alpha_1\)Is are strongly related. Strik-
ingly, however, and first indicated from sequence analysis of human PZP (Sotrup-Jensen et al., 1984c), rat aM (Gehring et al., 1987), and rat aL (Braciak et al., 1988), the stretches presumed to function analogously to the human aM bait region are dissimilar and of different length.

To further investigate the role of specific limited proteolysis in the activation of aMs, we have determined the bait region sequence of human PZP and report the sites of cleavage in that bait region and those of rat aM and aM, and the two isoforms of aL, by a set of proteinases differing in specificity. In conformity with results obtained earlier on human aM (discussed by Roberts and Hall (1983)), we find that each bait region of different primary structure (32–53 residues) contains one or more sites at which specific limited proteolysis leading to complex formation and activation of the internal thiol esters takes place. Some clustering of cleavage sites in two major areas in each bait region is apparent. The dissimilar bait regions suggest different evolutionary constraints for different parts of the aM structure.

**MATERIALS AND METHODS AND RESULTS**

**DISCUSSION**

**Amino Acid Sequence of PZP Bait Region**—The partial bait region sequence determined earlier (Sotrup-Jensen et al., 1984c) was completed by analyzing overlapping CNBr fragments and tryptic peptides obtained from PZP or the PZP-chymotrypsin complex (Figs. 1–3 and Table 1). The sequence of a 90-residue segment containing the bait region is shown in Fig. 4.

**Sequence Diversity of aM Bait Region**—The gross structure of the human aM subunit and the extent of overall sequence similarity among human aM and rat aM, aM, and aL are illustrated in Fig. 5. Scores of pairwise identity range from 75% for human aM versus rat aM to 55% for rat aM versus rat aL. In the four aM sequences shown, 41% of all residues are conserved (56% when chemically similar residues are included). The aMs evidently constitute a family of homologous proteins resulting from divergent evolution. The bait region sequence of each aM is the only major segment of dissimilar sequence (protein and DNA level). Fig. 6 shows an alignment of a 47-residue stretch containing the human aM bait region with corresponding stretches in PZP and the rat aMs including the two variants of rat aL. From this, we define the bait region of an aM as the stretch of highly variable sequence corresponding to the segment located between residues 666 and 706 in human aM flanked by segments of strongly conserved sequence. As shown in Fig. 6, the bait regions also differ in length, spanning 39 residues in human aM, 49 residues in human PZP, 53 residues in rat aM, 32 residues in rat aM, and 52 or 53 residues in rat aL variants 1 and 2, respectively.

It is likely that the evolutionary constraints of the bait regions, which constitute the segments involved in protease recognition, differ from those of the aM gross structure. This is reminiscent of the ovomucoid proteinase inhibitors (Laszlo et al., 1987) and the plasma proteinase inhibitors related to α-antitrypsin (Hill and Hastie, 1987). With regard to the evolution of the reactive sites and areas of protease contact, these protein families are thought to represent cases of positive Darwinian selection. The finding that the bait region sequence of rat aM is encoded as a separate exon indicates that the bait regions constitute elements of distinct genetic origin and that exon shuffling might be important in the evolution of aMs (Braciak et al., 1988).

**Structural Features of aM Bait Regions**—With the exception of PZP, whose bait region contains only 3 charged residues, the bait regions are highly charged (human αM, 9 residues; rat aM, 6 residues; rat aM, 8 residues; rat aL variant 1 and 2, 12 and 13 residues, respectively). Apart from the presence of one or more negative charges near the COOH-terminal boundary, there appear to be no common patterns of charges. About one-third to one-half of the residues are hydrophilic. Furthermore, apart from rat aM and rat aL variant 2, Gly residues and, with the exception of rat aM, Pro residues are abundant. In examining the sequences using the procedures of Chou and Fasman (1978) and Garnier et al. (1978) (data not shown), no common pattern of α-helix and β-sheet is indicated. Several of the bait regions contain clustered hydrophobic residues, and hydrophobic plots (Kyte and Doolittle, 1982) (Fig. 7) indicate the presence of a hydrophilic region separating two hydrophobic segments which largely correspond to the two major areas of proteolytic cleavage identified in each aM bait region (Fig. 6). The bait regions have been suggested to be irregular and flexible structures (Roberts and Hall (1983) and Sotrup-Jensen (1987))) in which even relatively hydrophobic stretches are readily accessible to cleavage (Fig. 6). It is presently not known whether the bait regions in spite of their sequence diversity assume a common gross structure.

Recent 1H NMR studies of the resonances of aromatic residues in human αM (Gettins and Cunningham, 1986; Arakawa et al., 1986) support the contention that its bait region is flexible. Although restrictions in motility do exist, residues 683–700 appear to constitute a highly flexible surface...
cleavage, which obviously constitutes the activating event, trypsin, azM-chymotrypsin, and rat cYzM-elastase, only one occurred. However, in most other aM-proteinase complexes, multiple cleavages were identified; and these could be the result of a single primary cleavage (activating event) followed by one or more secondary cleavages, or they could be the result of random cleavages, each giving rise to activation of the aM. Clear-cut examples of sequential cleavage are provided by PZP-chymotrypsin and rat aM-chymotrypsin, in which a single cleavage occurring in the NH2-terminal part of their bait regions is followed by secondary cleavages toward the COOH terminus see (“Results”). In contrast, trypsin apparently cleaves rat aM randomly at two nearby sites, as judged from the high rate of interaction between human aM and proteinases such as trypsin, chymotrypsin, and pancreatic and neutrophil elastases (overall second-order rate constant 5.3 x 10^7 M^-1 s^-1). In PZP-thermolysin and in rat aM-elastase, several adjacent or closely spaced cleavage sites, e.g. in PZP- elastase and PZP-thermolysin and in rat aM-papain, suggest that random cleavage may occur in many cases. The maximal distances between sites cleaved by the proteinases studied here are 20 residues (rat aM), 25 residues (human aM), 26 residues (rat aM), 31 residues (human PZP), and 39 residues (rat aM). Whether proteolytic cleavage may occur at any position within these segments is not known.

Localisation of Cleavage Site for Proteinases in Bait Region of Human PZP and Rat aM, aM, and aL variants 1 and 2. The aM bait regions are defined as the stretch of dissimilar sequence corresponding to residues 666–706 in human aM. cc, Clostridium histolyticum collagenase; fc, human fibroblast collagenase; tr, bovine trypsin; th, bovine thrombin; st, Streptomyces griseus trypsin; sb, Streptomyces griseus proteinase B; pe, porcine pancreatic elastase; cs, human cathepsin G; cs, calf chymosin; ct, bovine chymotrypsin; sp, S. aureus proteinase; pl, human plasmin; su, subtilisin Novo; he, human leucocyte elastase; tl, thermolysin; pa, papain. Data for cleavage sites in human aM were taken from Sottrup-Jensen, et al. (1981a) tr, pe, th, and pe), Hall et al. (1981) (tr and sp), Mortensen et al. (1981b) tr, st, sb, pa, ct, and su), Virca et al. (1983) (he and cs), and Arakawa et al. (1986) (tr). Data on cleavage sites for C. histolyticum collagenase and human fibroblast collagenase were taken from Sottrup-Jensen and Birkedal-Hansen (1989). The localization of cleavage sites in the rat aM variants for porcine pancreatic elastase is tentative (see “Results”). Gln-670 and Gln-671 in human aM (marked with asterisks) are accessible to reaction with plasma transglutaminase factor XIII, (Mortensen et al. 1981a). The areas of primary and secondary cleavage in human aM (residues 681–686 and 696–700, respectively) are indicated by (+ +).
constant for complex formation, >1 × 10^2 M"1 s"1) (Barrett and Salvesen, 1979; Christensen and Sottrup-Jensen, 1984; Virca and Travis, 1984; Bieth and Meyer, 1984), the bait region can serve as an excellent substrate. Qualitative data (see "Results")) indicate that the bait regions of FPZ and the rat αMMs may also be rapidly cleaved by several proteases. This suggests that segments of the bait regions are readily accessible at the surface of the αM structure (Roberts and Hall, 1983). However, for other proteases including thrombin, factor Xa, and urokinase, the human αM bait region is a relatively poor substrate (second-order rate constants, <1 × 10^2 M"1 s"1) (Downing et al., 1978; Ellis et al., 1982; Straight et al., 1985); and in some cases, e.g. factor XIIa, and activated complement factor C1s, no reaction has been demonstrated (Sim et al., 1979; Chan et al., 1985) and Dr. J. Arata, Y., and Ikai, A. (1986) Biochemistry 25, 6785-6789.

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EXPERIMENTAL

\(\alpha\)-Macroglobulin complexes were formed by incubating 1-2 mg of enzyme (4-10 \(\mu\)M) with different proteases at the following estimated molar ratios: 1.7; 0.19; 0; 0.5 (rat \(\alpha_{2}\)M); 1.0; 0.4; 1.0 (human \(\alpha_{2}\)M); and 1.0; 1.5; 0.0 (rat \(\alpha_{2}\)M and \(\alpha_{2}\)M) for 5 \(\mu\)M to 1 h as described in the Results section. In Table 2, \(\alpha_{2}\)M from rat serum used in these experiments was processed by extraction and purification methods described previously (Sottrup-Jensen et al. 1984). In most cases, the time needed for complete proteolysis of the bait region was controlled by using an excess of \(\alpha_{2}\)M relative to the protease to ensure that the protease concentration varied only slightly during the incubation period. As a result, the variation in the concentration of free active enzymes was small. Reactions were stopped by addition of a solution of chymotrypsin inhibitor and 50\% ethanol. The \(\alpha\)-macroglobulin complexes were separated by two-dimensional isoelectric focusing and SDS-PAGE on a 7.5\% gel. The gel was partially stained with Coomassie blue R-250 and sliced into 1-cm segments. A \(\alpha\)-macroglobulin reference sample was electrophoresed simultaneously for comparison. The gel segments were then placed in tubes containing formic acid and were heated at 90°C for 1 h. The resulting protein extracts were then lyophilized, redissolved in 100 \(\mu\)M SDS and 5\% mercaptopropanol, and analyzed by SDS-PAGE on a 7.5% gel. The electrophoresis system was similar to that used by Sottrup-Jensen et al. (1984a). The gels were stained with Coomassie blue R-250 and destained with 25\% acetic acid. The bands corresponding to the bait region were cut out and digested with chymotrypsin. The resulting peptides were subjected to amino acid analysis and trypsin digestion. The digests were then analyzed by HPLC as described previously (Sottrup-Jensen et al. 1984a).
RESULTS

Determination of the Bait Region Sequence of Human PEP

In order to determine the sequence of the PEP bait region PEP was degraded with CNBr and the N-terminal sequence of partially purified fragments assigned. As judged from the extent of sequence similarity between PEP and n.p. (98% in peptides covering nearly half of the PEP amino acids) this approach might identify a fragment which could contain its entire bait region sequence. One CN-bronube pool (experimeintal section) was subjected to 34 stages of DNase degradation and each fragment was RIA-identified. In the entire process of sequencing the major fragment was identified to that determined for residues 74-81 of PEP (Hoppenrath et al. 1984) while the minor fragment (CB) was judged to originate at a position corresponding to residues 61 of human PEP. However, while CB could not be separated from the accompanying fragments a 25-residue tryptic peptide (CT) corresponding to that initially found (Hoppenrath et al. 1984) was identified (Fig. 2) and the sequence of its N-terminal 61 residue determined (Table 1). In order to overlap the sequence of this peptide with stretches already isolated chromatographically of the PEP bait region, tryptic peptides of the tryptic digest were examined for their capacity to react with anti-CT sera. Peptides YNI(58)N(60)K(N(61)N) and N(61)K(N(62)N) of the tryptic digest of CB were isolated pure by passage through a 5 ml peptide CB column (Fig. 2) and the sequences of these peptides determined (Table 1). Class II sequence CPA was determined earlier and which originated at a position corresponding to residue 10 in human PEP, from the digest which produced CB-1. Peptide CB-1 corresponding to the N-terminal 12 residues of CB was isolated chromatographically via its capacity to react with anti-CT sera. Peptide T(5)CT(5)M(6)N(7)K(8) of the tryptic digest of CB was separated from the major tryptic sequence while the sequences of CB, TI, and CT were consistent with their position in CB (not shown).

The sequence of 193 residues of CB including the bait region of PEP is shown in Fig. 4 and represents the sequence contained in the peptide T1(23)CT(24)N(25)A(25)C(25) of the tryptic digest of CB. Peptide T1 was identified by sequence from its capacity to react with antisera raised against T1. Residue 24 of T1 corresponds to a methionine residue. The sequence of T1(23)CT(24)N(25)A(25)C(25) is shown in Fig. 4. Residues 24 was determined by its capacity to react with anti-Met sera and with T1 as determined by sequence analysis. Residue 23 was determined from the sequence of T2 (not shown) which corresponds to residues 67-73 of human PEP. The sequence of T1(23)CT(24)N(25)A(25)C(25) is shown in Fig. 4. Residues 24 was determined by its capacity to react with anti-Met sera and with T1 as determined by sequence analysis. Residue 23 was determined from the sequence of T2 (not shown) which corresponds to residues 67-73 of human PEP. The sequence of T1(23)CT(24)N(25)A(25)C(25) is shown in Fig. 4. Residues 24 was determined by its capacity to react with anti-Met sera and with T1 as determined by sequence analysis.

Fig. 1. HPF separation of tryptic peptides originating from a pool containing CB. A column of Bio-Rex 7 cm x 25 cm was equilibrated with 10 mm ammonium acetate, pH 7.0 and eluted with a gradient of 0.1 M ammonium acetate. Fractions 13-20, containing peptides of CB, and 24-30 containing peptides of CB-1 were identified by N-terminal sequence analysis. Elution was carried out at a flow rate of 2 ml/min at 60°C. The sequence of CB was determined by matching the elution of peptides of CB and CB-1 with the sequence of peptides originating from the fraction of CB and CB-1. The sequence of CB was determined by matching the elution of peptides of CB and CB-1 with the sequence of peptides originating from the fraction of CB and CB-1.

Fig. 2. Ion-exchange chromatography of Na-carboxyanhydridated medium-sized CNBr fragments from PEP. A column of Bio-Rex 7 cm x 25 cm was equilibrated with 10 mm ammonium acetate, pH 7.0 and eluted at a flow rate of 2 ml/min at 60°C. The sequence of peptides CB and CB-1 was determined by matching the elution of peptides with the sequence of peptides originating from the fraction of CB and CB-1.

Fig. 3. HPLC separation of a pool containing low molecular weight CNBr fragments from PEP-chymotryptic complex. A column of Bio-Rex 7 cm x 25 cm was equilibrated with 10 mm ammonium acetate, pH 7.0 and eluted at a flow rate of 2 ml/min at 60°C. The sequence of peptides CB and CB-1 was determined by matching the elution of peptides with the sequence of peptides originating from the fraction of CB and CB-1.
In order to map the sites of bait region cleavage a representative set of proteins including trypsin, thrombin, chymotrypsin, pancreatic elastase, 2,4-dinitrophenyl, and chymotrypsinogen was used. These proteins differ greatly in their substrate specificity and represent three of the four commonly recognized classes of proteases: trypsin-like, thrombin-like, and chymotrypsin-like enzymes.

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Determination of the sites of cleavage by different proteases

The results of the study are shown in Table 2-3. In almost every case the My-terminal sequence of the covalently bound proteins was clearly seen. The yield of these sequences was approximately 10% of the yield of the original My-terminal sequences of the bait as expected from the high level of contaminant binding seen for each bait region. Three independent preparations of human bait region cleavage sites have been obtained from Table 2-3.

Upon incubation with chymotrypsin two new My-terminal sequences, 1 major and 2 minor, were seen (Table 2A). In an experiment where the incubation period was 5 min only one sequence was found (not shown). The ability of chymotrypsin to cleave the bait region was the same in all five preparations of human bait region cleavage sites that were examined (Table 2-3).

In P5-elastase complex three major My-terminal sequences were present corresponding to bait region cleavage sites following a hydrophobic residue (Table 2A). These sites probably represent the major cleavage sites since following a hydrophobic the native bait region cleavage sites for 5 min none of these sequences were recognized due to extensive fragmentation.

Although the sequence of protease cleaves these My-proteins very slowly, and when present in excess gives rise to extensive fragmentation (Smith et al., 1974), a specific cleavage site is the bait region preceding the first amino acid of the human gaj (Table 3C). The extent of cleavage was less than 5% even after 65 min incubation at 37°C. In P5-elastase complex a major new My-terminal sequence corresponding to bait region cleavage was present (Table 3A) and the sequence was also identified in Table 3B (Table 3C). The presence of this new My-terminal sequence was also identified in Table 3B (Table 3C). The presence of this new My-terminal sequence was also identified in Table 3B (Table 3C). The presence of this new My-terminal sequence was also identified in Table 3B (Table 3C).
TABLE 1. Results of N-ethylmaleimide sequence analysis of complexes between human Fcy and different proteins.

| Protein | Conditions | PTH amino acids recovered | N. | M. |
|---------|------------|---------------------------|----|----|
| Fcy | 50°C, pH 7.4 | 1.0 | 1.0 |
| | 37°C, pH 7.4 | 0.9 | 0.9 |
| | 25°C, pH 7.4 | 0.8 | 0.8 |

TABLE 2. Results of N-ethylmaleimide sequence analysis of complexes between rat a-M and different proteins.

| Protein | Conditions | PTH amino acids recovered | N. | M. |
|---------|------------|---------------------------|----|----|
| a-M | 50°C, pH 7.4 | 1.0 | 1.0 |
| | 37°C, pH 7.4 | 0.9 | 0.9 |
| | 25°C, pH 7.4 | 0.8 | 0.8 |

TABLE 3. Results of N-ethylmaleimide sequence analysis of complexes between rat a-M and different proteins.

| Protein | Conditions | PTH amino acids recovered | N. | M. |
|---------|------------|---------------------------|----|----|
| a-M | 50°C, pH 7.4 | 1.0 | 1.0 |
| | 37°C, pH 7.4 | 0.9 | 0.9 |
| | 25°C, pH 7.4 | 0.8 | 0.8 |
TABLE 4. Results of NH2-terminal sequence analysis of complexes between rat α2 and different proteases.

| Conditions | Original NH2-terminus | New NH2-terminus |
|------------|-----------------------|------------------|
| A. Rat α2-trypsin (1:1.5 mol/mol, 20 s) | Set - Ala - Pro - Glu - Lys - Pro - His - Tyr | (1.3) (1.2) (1.7) (1.5) (0.9) (1.5) |
| B. Rat α2-chymotrypsin (1:1.5 mol/mol, 10 s) | Gly - Ile - Pro - Ala - Ala - Tyr - His - Leu | (2.3) (2.2) (2.8) (2.3) (2.6) (2.4) |
| C. Rat α2-elastase (1:1.5 mol/mol, 10 s) | His - Leu - Val - Ser - Glu - Ser - His - Met | (0.8) (2.6) (2.8) (2.3) (2.2) (2.2) |
| D. Rat α2-nor-terminus (1:1.5 mol/mol, 7 min) | NH2-termini | Gly - Ile - Pro - Ala - Ala - Val |

TABLE 5. Results of NH2-terminal sequence analysis of complexes between rat α1,2 and different proteases.

| Conditions | Original NH2-terminus | New NH2-terminus |
|------------|-----------------------|------------------|
| A. Rat α1 variant 1-H_murinus proteases (1:0.25 mol/mol, 15 min) | Ser - Ala - Gly - Arg | (1.4) (0.5) (1.7) (0.6) (0.6) |
| B. Rat α1 variant 1-H_murinus proteases (1:0.25 mol/mol, 10 min) | Gly - Ile - Pro - Ala - Ala - Tyr | (1.4) (0.5) (1.7) (0.6) (0.6) |
| C. Rat α1 variant 1-chymotrypsin (1:0.30 mol/mol, 30 s) | His - Leu - Val - Ser - Glu - Ser - His - Met | (0.8) (2.6) (2.8) (2.3) (2.2) (2.2) |
| D. Rat α1 variant 1-chymotrypsin (1:0.30 mol/mol, 30 s) | NH2-termini | Gly - Glu |

Conditions of complex formation are indicated for each experiment.