Degradation of type I and II collagen by human $C\bar{1}s$

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The activated first component of human complement, $C\bar{1}s$, was shown to cleave type I and II collagen and gelatin. The proteolytic activity was heat labile and was inhibited by a monoclonal antibody (M241) which recognized light chain of active human C1s or by a serine protease inhibitor, DFP, but not by a chelating agent.

$C\bar{1}s$; Collagen type I; Collagen type II

1. INTRODUCTION

We have purified a calcium-dependent serine proteinase (CASP) from the conditioned medium of hamster embryo fibroblasts, Nil2C2 cells [1] with high metastatic and phagocytotic activities [2]. CASP had been shown to degrade several components of extracellular matrix. Nucleotide sequence of the complementary DNA of CASP [3] was found to have a homology with that of human C1s, whose complete nucleotide sequence was reported in several studies [4–6]. We also found that CASP cleaved human C2 and C4 to form C3 convertase. Monoclonal antibodies against human C1s recognized CASP and the converse was the case [7]. Moreover, an immunohistochemical examination of CASP revealed that chondrocytes of hyaline cartilage were highly reactive to a monoclonal antibody against CASP [8]. Therefore, we decided to investigate the proteolytic activity of activated human C1s ($C\bar{1}s$) towards extracellular matrix proteins such as collagen and fibronectin, which had been shown to be cleaved by CASP [1].

2. MATERIALS AND METHODS

2.1. *Purification of human $C\bar{1}s$*

$C\bar{1}s$ was purified as described by Gigli [9] with modifications [10]. Briefly, 1 vol. of fresh human serum was added to 4 vols of distilled water containing 5 mM $CaCl_2$ (pH 7.4). The precipitate was resuspended in 10 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl and 5 mM $CaCl_2$, and applied on Sepharose 6B column. The fractions with $C\bar{1}$ hemolytic activity were then separated on a DEAE-Sephadex A-50 column with a linear gradient of NaCl (0.15–0.25 M) in 10 mM Tris-HCl buffer (pH 7.8). $C\bar{1}s$ was finally purified with Sephadex G-200 column.

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Abbreviations: CASP, calcium-dependent serine proteinase; AGLNE, acetyl-Gly-L-Lys-naphthyl ester; DFP, diisopropyl fluorophosphate

2.2. *Preparation of monoclonal antibody against human $C\bar{1}s$*

A monoclonal antibody against human $C\bar{1}s$ was obtained by immunizing (BALB/c × C57BL/6) F_1 mice with purified human $C\bar{1}s$ as reported before [10]. Clones were screened for the ability to inhibit C1s activity by microplate hemolytic ELISA assay. An antibody, M241, which recognized the activated form of C1s but not the inactive one [10] was used in the present study.

2.3. *Proteolytic assay of human $C\bar{1}s$*

Five μg of type I (Vitrogen 100, Flow Lab., VA), mouse type II collagen, gelatin (Sigma Co., MO), fibronectin (Extraco Nobel Indust., Sweden) or laminin were incubated with 1 μM human $C\bar{1}s$ at 37°C overnight in 20 μl of 100 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl and 5 mM $CaCl_2$. To examine effects of monoclonal antibody (M241), diisopropyl fluorophosphate (DFP) (Sigma Co., MO) or EDTA on the proteolytic activity, the reagents were incubated with $C\bar{1}s$ for 30 min at room temperature followed by overnight incubation with the substrates. After the incubation, samples were electrophoresed on 7.5% SDS-polyacrylamide gel (SDS-PAGE) under reducing conditions. Effects of C1s inhibitors were quantitated by densitometric scanning of the gel using Soft Laser Scanning Densitometer model SLR-2D/1D (Biomed Instr., CA).

2.4. *Esterase assay*

The esterase activity of human C1s was measured by utilizing synthesized peptides, acetyl-Gly-L-Lys-naphthyl ester (AGLNE), as described [11] with minor modifications. Human $C\bar{1}s$ (0.1 μg) and 6.3 nmol of AGLNE, were incubated in 50 μl of 10 mM Tris-HCl buffer (pH 7.4) and 6.3 μl of 1% Fast violet B salt were added. The wine color developed was quantitated at 515 nm with a spectrophotometer model DU-65 (Beckman Instr., CA).

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3. RESULTS AND DISCUSSION

The cDNA encoding for CASP contains a 5′ noncoding region, 2085 nucleotides coding for a polypeptide precursor of 695 amino acids containing 63 nucleotides coding for a signal peptide and a 3′ noncoding region. The cDNA of human C1s consists of 2064 nucleotides including 45 bases coding for a signal peptide. Homology of nucleotide sequences of coding regions between CASP and human C1s was 81.0%, but homologies were detected neither at 3′ nor at 5′ untranslated regions (data not shown). The deduced amino acid sequence of CASP was compared with that of human C1s (Fig. 1). The leader sequence of CASP is 6 amino acids longer than that of human C1s. Both CASP and human C1s are cleaved between Arg and Ile to be converted to active forms (Fig. 1, arrowhead). The heavy chains are highly homologous to each other (81.0%) including alignment of cysteine residues and mouse epidermal growth factor precursor domain. The light chains which contain the catalytic site of serine protease were less homologous than the heavy chain (68.3%). Particularly, there are several regions where distinct amino acids cluster near the active center.

In addition to a strong homology of cDNA between CASP and human C1s, they share similarities in biochemical and immunological properties. Those observations indicate that CASP is hamster C1s [7]. Therefore, we have examined if human $\overline{C1s}$ degrades extracellular matrix proteins which are hydrolyzed by

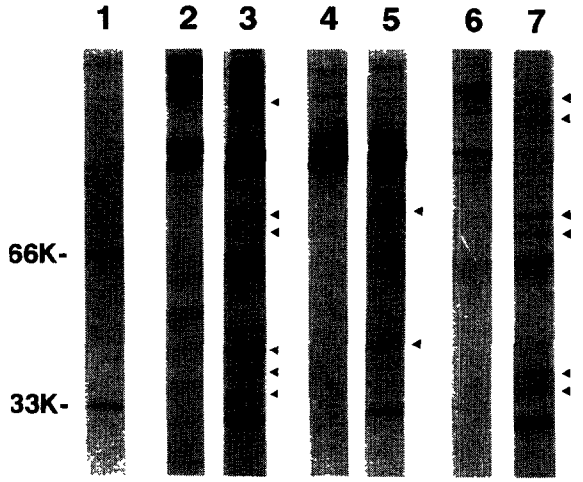

Fig. 2. Degradation of type I and II collagen and gelatin by human $\overline{C1s}$. Five μg of type I, II and gelatin were incubated with 1 μM of human C1s at 37°C overnight in 20 μl of 10 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl and 5 mM $CaCl_2$ followed by electrophoresis on 7.5% SDS-PAGE and Coomassie blue staining. Arrowheads show degradation products. (Lane 1) Human $\overline{C1s}$; (lanes 2,3) type I collagen; (lanes 4,5) type II collagen; (lanes 6,7) gelatin; (lanes 3,5,7) substrates with human $\overline{C1s}$.

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  1  MGKSSEAWCIVLFSVFASFSAEPTMHGEILSPNYPQAYPNEMEKTWDIEVPEGFGVRLYF
  1        M.......LL.WVY.....Y.............S.V..S.........Y.IH...

 61  THLDMELSENCEYDSVQIISGGVEEGRLCGQRTSKNANSPIVEEFQIPYNKLQVIFRSDF
 55  ....I......A.........DT.........S.N.PH........V.........K...

121  SNEERFTGFAAYYAAIDVNECTDFTDVPCSHFCNNFIGGYFCSCPPEYFLHDDMRNCGVN
115  .............V.T.I......V.............................K.....

181  CSGNVFTALIGEISSPNYPNPYPENSRCEYQILLEEGFQVVVTIQREDFDVEPADSQGNC
175  ...D.........A.....K............R..K.......LR.......A...A...

241  QDSLLFAAKNRQFGPFCGNGFPGPLTIETHSNTLDIVFQTDLTEQKKGWKLRYHGDPIPC
235  L...V.V.GD.....Y..H......N...K..A...I......G.............M..

301  PKEITANSVWAPEKAKYVFKDVVKISCVDGFEAVEGNVGSTFFYSTCQSNGQWSNSRLRC
295  ...D.P....E.A......R...Q.T.L....V...R..A.S.........K....K.K.

361  QPVDCGIPEPIQNGKVDDPENTLFGSVIHYSCEEPYYYMEHAEHGGEYRCAANGSWVNDE
355  .........S.E....E...S.......R.T.........NGG-....H..G......EV

421  LGIELPKCVPVCGVPTEPFRIQQRIFGGFPAKIQSFPWQVFFEFPRAGGALIGEHWVLTA
414  ..P............R...EEK...I..SD.D.KN.......DN.W......N.Y.....

481  AHVVEGNSDPSMYVGSTSVRMENLANVQKLTTDRVIIHPGWKPGDDLSTRTNFDNDIALV
474  .......RE.T.........QTSR..KSKM..PEH.F......LLEVPEG...........

541  RLKDPVKMGPTVSPICLPGTSSEYEPSEGDLGLISGWGRTERRNIVIQLRGAKLPVTSLE
534  ......................D.NLMD.............K.DRAVR.KA.R...AP.R

601  KCRQVKEENPKARADDYVFTSNMICA-GEKGVDSCQGDSGGAFALPVPNVRDPKFYVAGL
594  ..KE..V.K.T.D.EA....P.....G....M...K.........VQD..DK-T...A...

660  VSWGKKCGTYGIYTKVKNYKDWILQTMQENSVPSQD
653  ....PQ.....L..R....V...MK......T.RE.
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Fig. 1. Homology of amino acid sequences between CASP and human C1s. · = identical residues; – = deletion; ▬ = signal peptide; ▼ = the cleavage site between heavy and light chain; ★ = the active sites of serine protease; ■ = sites of possible N-linked glycosylation.

Table I

Effects of proteinase inhibitors and M241 on $\overline{C1s}$ activity

Inhibitors	Activity (% control)			
	Substrates:	Collagen		AGLNE
		Type I	Type II	
No addition		100	100	100
M241		15.0	20.2	23.4
MIg		92.1	89.4	187.6
DFP		0	0.1	7.4
EDTA		113.9	103.4	78.6
Heat treatment (56°C, 30 min)	2.2	ND*	0.9	

Type I or II collagen (5 μg) was incubated with human $\overline{C1s}$ (1 μg) with or without an inhibitor. M241 or mouse IgG (MIg) was added at the 1:2 antibody/enzyme mol ratio, and DFP or EDTA was added at the concentration of 1 mM or 5 mM, respectively. M241 or MIg was used at the 5:1 antibody/enzyme mol ratio to examine the effect on the esterase activity. * ND, not done

CASP [1]. Type I and II collagen and gelatin were shown to be degraded by human $\overline{C1s}$ (Fig. 2). There were several digestion products in the incubation mixture of $\overline{C1s}$ and type I collagen. $\overline{C1s}$ split type II collagen of mouse into two fragments of 2:1 ratio in length. The same fragmentation was observed when type II collagen of chicken or monkey was used. Those were different from degradation products of typical type I or II collagenase [12]. Because the collagenases cleave collagen 75% and 25% in length and split Gly-Ile or Gly-Leu bond. On the other hand, $\overline{C1s}$ hydrolyzes the carboxyl-terminal side of Lys, Arg or Tyr [13–15]. Unlike collagenases, the activity of $\overline{C1s}$ was completely blocked by a serine protease inhibitor, DFP, but not by EDTA (Table I). The monoclonal antibody, M241, inhibited the esterase and collagenase activities. The esterase and proteinase activities were both heat labile.

Different from CASP, human $\overline{C1s}$ did not require divalent cations nor did it degrade fibronectin or laminin (data not shown). Human $\overline{C1s}$ has been reported to hydrolyze tyrosine ester bond [13,14] besides that of lysine and arginine. However, CASP did not hydrolyze tyrosine ester [1]. These differences in substrate specificity might be caused by the differences in amino acid sequences between human and hamster C1s near the active center.

C1s is known as a subcomponent of the first complement, which reacts at the first step of classical complement pathway. Recently, however, Hakan et al. reported that human $\overline{C1s}$ cleaved human major histocompatibility complex (MHC) class I antigen [16,17]. They speculate that C1s might play an important role in the regulation of the immune response. The findings that C1s degraded type I and II collagen and that it was detected in chondrocytes of hyaline cartilage by immunohistochemical assay, suggest that C1s might participate in the metabolism in cartilage matrix. It is also very interesting to know if C1s is involved in pathogenesis of rheumatoid arthritis (RA). This possibility could be supported by the observation by Boisscer et al. who reported that the sera of patients suffering from RA showed higher titer of antibodies to denatured type II collagen than to native one [18].

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