Volume 223, number 2, 352–360 FEB 05250 November 1987

A secretable serine proteinase with highly restricted specificity from cytolytic T lymphocytes inactivates retrovirus-associated reverse transcriptase

Hans-Georg Simon, Ulli Fruth, Michael D. Kramer* and Markus M. Simon

Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg and **Onkologisches Labor der Universitätsklinik, Im Neuenheimer Feld, D-6900 Heidelberg, FRG*

Received 11 September 1987

TSP-1, a murine T cell specific proteinase, is expressed in cytolytic T lymphocytes and secreted upon their interaction with antigen bearing target cells. In searching for possible extracellular substrates of the enzyme in the physiological environment of cytolytic effector cells, we have investigated the proteolytic activity of TSP-1 on retroviral proteins. It is shown that reverse transcriptase derived from the retrovirus Moloney murine leukemia virus is inactivated by TSP-1 via limited proteolysis. The data suggest the possibility that cytolytic T lymphocytes are able to interfere with retroviral replication by secreting a serine proteinase which degrades viral proteins.

Serine proteinase; Reverse transcriptase; Virus replication; (Cytolytic T cell)

1. INTRODUCTION

Cytolytic T lymphocytes play an important role in the host defense of viral infections. The findings that specific CTLs are able to lyse virus-infected syngeneic cells in culture [1] and to confer protection against lethal viral infections in syngeneic mice [2–4] led to the concept that CTLs control viral replication in vivo by direct cytolysis of infected cells. However, direct evidence on the importance of CTL-mediated cytolysis in the control of viral infections is still lacking. In addition, studies demonstrating secretion of lymphokines [5], including IFNγ [6,7], by CTLs suggested the possibility of direct or indirect anti-viral activity exerted via soluble factors. Recently we [8,9] and others [10–12] have described a serine proteinase/serine esterase(s), respectively, isolated from cloned murine CTLLs. The T cell-specific serine proteinase, termed TSP-1 [8] or synonymously granzyme A [10], or SE1 [12], was shown (i) to be a disulfide-linked dimer with a molecular mass of 60 kDa, (ii) to have a highly restricted specificity [8], (iii) to be inducible in both T cell subsets – Lyt-2$^+$ and L3T4$^+$ – [13,14], (iv) to be expressed in all CTLLs and in some THLs tested so far [9], (v) to be associated with cytoplasmic granules of CTLs [10,12,15], and (vi) to be released into the extracellular space during CTL-target cell interaction [11,14,16]. Previous functional studies using the purified enzyme and a TSP-1 specific inhibitor have indicated that TSP-1

Correspondence address: M.M. Simon, Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg, FRG

Abbreviations: TSP-1, T cell specific proteinase-1; CTL, cytolytic T lymphocyte; TH, T helper cells; CTLL, CTL line; THL, TH line; IFNγ, interferon-γ; MoMuLV, Moloney murine leukemia virus; RT, reverse transcriptase; RT_{MoMuLV}, reverse transcriptase from MoMuLV; PMSF, phenylmethylsulfonyl fluoride; PFR-CK, H-D-Pro-Phe-Arg-chloromethylketone; PFR-NA, PFR-nitroanilide

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

is involved in the process of cytolysis [16], in the control of the humoral immune response [8] and in lymphocyte extravasation [17,18]. In searching for possible target structures of the secreted TSP-1 we have investigated whether viral proteins may serve as substrates for the T cell-specific enzyme. We now communicate that the enzyme RT associated with the retrovirus MoMuLV is inactivated by TSP-1 via limited proteolysis. Together with the finding that TSP-1 is specifically released from effector cells upon contact with target cells these results suggest that beside their cytolytic potential CTL might be able to control replication of retroviruses in vivo by a serine proteinase which degrades relevant viral structures.

2. MATERIALS AND METHODS

2.1. *Materials*

Chromogenic substrates were obtained from either Bachem AG (A-16056, A-13102, A-16071; Bubendorf, Switzerland), AB Kabi Peptide Research (S-2302, S-2444, S-2266, S-2288, S-2251, S-2586; München, FRG) or from Boehringer Mannheim (Chromozymes PL, TR4, TH; FRG). Tissue kallikrein (791164) was from Boehringer Mannheim, pancreatic trypsin from Merck Darmstadt (FRG) and plasma kallikrein (K 3126) from Sigma (München, FRG).

The enzyme inhibitors PMSF (P-7626) and PFR-CK were from Sigma, and Bachem, respectively.

All other chemicals of highest purity available were from Roth (Karlsruhe, FRG), Merck, Pharmacia (Freiburg, FRG) and Sigma. [^{3}H]DFP and [α-^{32}P]dATP were from Amersham (Braunschweig, FRG).

2.2. *Purification of TSP-1*

Purification of TSP-1 from a long term culture CTLL 1.3E6 [19] was achieved as follows: 2–3 × 10^9 cells of CTLL 1.3E6 ('aged killer' AK of C57BL/6 origin with specificity for P815 target cells) were disintegrated in lysis-buffer (0.01 mM Tris-HCl, pH 7.5, 0.1% Triton X-100) at 5 × 10^7 cells · ml^{-1} for 1 h on ice. Particulate material was removed at 2500 × *g* for 15 min. The resulting supernatant was saturated with 60% ammonium sulfate $(NH_4)_2SO_4$, stirred for 2 h on ice. Afterwards the sample was centrifuged at 40000 × *g* for 30 min and the pellet discarded. The supernatant was dialysed overnight against 0.1 M Tris-HCl, pH 8.5, and then loaded onto an arginine-Sepharose column (10 ml packed volume, Pharmacia) previously equilibrated with 0.1 M Tris-HCl, pH 8.5. The column was washed with the same buffer, followed by 0.5 M arginine in 0.1 M Tris-HCl, pH 8.5. Eluted fractions (1 ml) were monitored by assaying 10 μl aliquots for amidolytic activity on PFR-NA. Protein was determined in each fraction by the method of Bradford [20]. 0.5 M arginine eluted enzyme activity as a single peak, whereas practically no amidolytic activity could be detected in the pass-through. Active fractions (representing 15% of the initial activity) were pooled, dialysed against 0.1 M Tris-HCl, pH 7.5, and loaded onto an anion-exchange column (Mono Q HR 5/5, 17-0547-01 Pharmacia) previously equilibrated in 0.1 M Tris-HCl, pH 7.5, and connected to a FPLC unit. Protein was eluted with a linear gradient of 1 M KCl in 0.1 M Tris-HCl, pH 7.5 (0–100%). Fractions (1 ml) were collected and aliquots of each fraction tested for amidolytic activity on PFR-NA and protein as described above (a). Fractions with highest enzyme activity (between 150 and 250 mM KCl) representing 0.6–3% of the initial activity were pooled, dialysed against 0.1 M Tris-HCl, pH 7.5, and stored frozen. For analysis by gel electrophoresis, 20 μl of the purified enzyme preparation were mixed with 20 μl of sample buffer containing 20 mM dithiothreitol (DTT) and 5% SDS and were heated to 95°C for 5 min. Subsequently, 20 μl of the sample were run on a 12.5% Laemmli gel [21] and silver stained. Alternatively, 20 μl of the sample were affinity labeled by incubation with 1 μl (= 5 μCi; spec. act. 62.8 mCi/mmol) of [^{3}H]DFP (Amersham) for 2 h at 37°C, boiled in SDS buffer in the presence of DTT and run on an SDS gel as described before. After electrophoresis, the gels were treated with Enlightning (New England Nuclear), dried and subjected to fluorography with Kodak X-Omat AR films at −70°C using Quanta III intensifying screens.

2.3. *Assay for amidolytic activity*

Enzyme preparations were tested for amidolytic activity as follows: aliquots of test samples (in 100 μl) were mixed with 100 μl of the individual chromogenic substrate (3 × 10^{-4} M) in 100 mM

Tris-HCl, pH 8.5, in microtiter plates (Nunclon, 96-well multidish; Nunc, Wiesbaden, FRG). The enzyme activity was tested after 1 h at 37°C at 405 nm by spectrophotometric reading of the chromophore products in microtiter wells. The molar absorption coefficient is 10.4 l/mol per cm for 4-nitroaniline at 405 nm. An absorbance of 0.01 was defined as 1 unit of amidolytic activity. TSP-1 was used at 75 ng, the commercially available t-kallikrein at 20 ng, p-kallikrein at 5 μl of stock solution and pancreatic trypsin at 10 ng per well on the indicated chromogenic substrates. The enzyme activity was 125 U/μg for TSP-1, 800 U/l μg t-kallikrein, 96 U/10 μl p-kallikrein and 530 U/μg pancreatic trypsin on the model peptide H-D-Pro-Phe-Arg-NA.

2.4. *Assay for proteolytic activity*

Hydrolysis of the protein substrate azocoll by three related enzymes was determined as follows: the incubation mixture contained 5 mg azocoll (19493, Calbiochem, Frankfurt, FRG) and 1 μg of either TSP-1 or t-kallikrein or 25 ng of pancreatic trypsin in a total volume of 1.1 ml of 0.1 M Tris-HCl, pH 8.5. After 1 h incubation at 37°C changes in absorbance at 520 nm were recorded in the clear filtrate, previously freed from the insoluble azocoll as described [22]. Blank values, obtained in the absence of enzyme were subtracted in each case.

2.5. *Reverse transcriptase activity assay*

100 U cloned MoMuLV RT (= 4 pmol = 1.1 μg protein; product information BRL, Karlsruhe, FRG) were incubated with/without serine proteinases for 2 h in 20 mM Tris-HCl, pH 8.0, at 37°C. Control reactions containing the protease inhibitor PMSF (3 mM) were preincubated with the protease alone for 30 min at 37°C before adding the reverse transcriptase. Finally, reverse transcriptase activity was tested in a reaction mixture (50 μl) containing 50 mM Tris-HCl, pH 7.5, 75 mM KCl, 3 mM $MgCl_2$, 30 mM β-mercaptoethanol, 40 U RNAsin (Biotek), 0.5 mM each of dCTP, dGTP, dTTP (Pharmacia) and 1 μCi [α-^{32}P]dATP (Amersham, 800 Ci/mmol), 10 μg/ml oligo(dT)$_{12-18}$ (Pharmacia) and 100 ng rabbit globin mRNA (BRL) according to Maniatis et al. [23]. The ^{32}P-labeled cDNA products were run on a 5% polyacrylamide gel containing 5 M urea and visualized by autoradiography.

2.6. *Analysis of proteolytic degradation products by SDS-PAGE*

100 U cloned MoMuLV RT (BRL) were incubated with/without proteases for 2 h in 20 mM Tris-HCl, pH 8.0, at 37°C. Control reactions containing the protease inhibitors PFR-CK (1 mM) or PMSF (3 mM) were preincubated with the protease alone for 30 min at 37°C before adding the reverse transcriptase. Degradation products were separated by electrophoresis on a PhastSystem equipment (Pharmacia, Freiburg, FRG) on a 10–15% gradient SDS-polyacrylamide gel after denaturation of the samples at 95°C for 5 min in 10 mM dithiothreitol (DTT), 2.5% SDS. Proteins were visualized by silver staining.

3. RESULTS

3.1. *General remarks*

Previous studies have shown that TSP-1 is a serine proteinase with a remarkably restricted specificity for the chromogenic substrate PFR-NA [8,9]. The finding that model peptides, synthesized according to the AA sequences at the cleavage site in native proteins, may imitate the structure following the bond split by the enzyme in natural substrates ([24] and Wolf, H.D., personal communication) suggested that TSP-1 may recognize the same or a similar array of AA sequences on proteins as well. This is also substantiated by a study reporting the identification and isolation of a hormone processing proteinase from yeast by applying short model peptides, representing those sequences of the precursor protein, where cleavage is thought to occur in vivo [25].

Based on these observations we used the peptide sequence PFR, which is most readily recognized by TSP-1 among the chromogenic substrates tested so far, to search for proteins containing this tripeptide. By screening the Dayhoff, Doolittle and EMBL protein sequence databanks it became obvious that many proteins encoded by DNA and RNA viruses including retroviral core and envelope proteins and polymerases (reverse transcriptases) from AKR MuLV, AKV MuLV, Rauscher spleen focus-forming virus and from human immunodeficiency virus (HIV) as well as the trans-activating transcriptional regulatory protein of HTLV-I and the putative protease of

HTLV-II contain PFR or very similar AA sequences with L-arginine in position P_1.

Although we realize that this finding may be accidental and merely due to the composition of the databanks we felt it sufficiently worthwhile – also in view of the important role of CTLs in the control of virus replication in vivo – to investigate whether TSP-1 is able to degrade and/or inactivate retroviral proteins, in particular RT, an enzyme essential for retroviral replication [26].

3.2. *Comparison of substrate specificity of TSP-1 and related serine proteinases*

TSP-1 used in this study was a homogeneous preparation as revealed by a single band with a molecular mass of 60 kDa (under nonreducing conditions) and by a broad band with a molecular mass of 30–35 kDa (under reducing conditions) (fig.1, and [15]). In addition, both molecular species (nonreduced and reduced) coincide with molecules labeled with [^{3}H]DFP, a specific affinity ligand for serine proteinases (fig.1).

For a meaningful interpretation of the functional data shown here it was of importance to include in the experimens in addition to TSP-1 other serine proteinases, with either highly restricted or less restricted proteolytic activities such as tissue (t)- and plasma (p)-kallikrein or pancreatic trypsin, respectively. Table 1 shows a comparison of the amidolytic activities of the enzymes. TSP-1, and both t- and p-kallikrein are highly specific for arginine amides and are restricted to few substrates whereas pancreatic trypsin has a much less restricted specificity. When tested on the protein substrate azocoll, an insoluble, collagen-dye conjugate [22], TSP-1 and t-kallikrein only showed marginal proteolytic activity whereas the same substrate was much more rapidly (>100-fold) hydrolysed by pancreatic trypsin (table 2). These results again emphasize the highly restricted proteolytic activities of TSP-1 as well as those of t- and p-kallikrein [28,29].

3.3. *Effect of TSP-1 on the polymerase activity of RT*

For the following study we used a commercially available preparation of recombinant RT from MoMuLV which is often taken as a prototype for mammalian RNA tumor viruses. Preparations of purified TSP-1, of t- and p-kallikrein and of pancreatic trypsin were adjusted to protein concentrations expressing similar amidolytic activity on the model peptide substrate PFR-NA and were incubated with aliquots of RT_{MoMuLV}. Alternatively, the enzyme preparations were pretreated with proteinase inhibitors PMSF and PFR-CK and then incubated with RT_{MoMuLV}. PMSF is a class specific inhibitor for most serine proteinases, including TSP-1 [8], t- and p-kallikrein and pancreatic trypsin [30]. PFR-CK had been synthesized as a specific inhibitor for TSP-1 according to the AA target sequence recognized by this serine proteinase on model peptide substrates [15,16]. Residual activity of RT_{MoMuLV} was tested in a reverse transcriptase assay system containing rabbit globin mRNA and radioactivcly labeled nucleotides. Newly synthesized ^{32}P-labeled cDNA

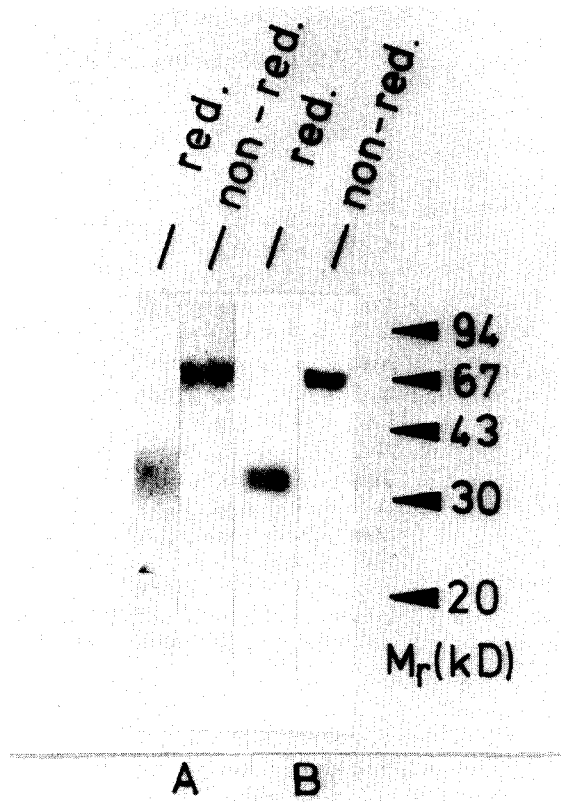

Fig.1. SDS-PAGE analysis of purified TSP-1. The purified enzyme was incubated with [^{3}H]DFP before treatment with SDS as described in section 2. (A) Fluorogram of [^{3}H]DFP-labeled protein. (B) Proteins stained with silver nitrate.

Table 1

Substrate specificity of purified proteinases on model peptide substrates

Substrates tested	TSP-1	Percent relative actvity of		
		Kallikrein (tissue)	Kallikrein (plasma)	Trypsin (pancreatic)
H-D-Pro-Phe-Arg-NA	100	62	100	12
Bz-Phe-Val-Arg-NA	26	0	0	42
Tos-Gly-Pro-Arg-NA	4	0	41	100
H-D-Ile-Pro-Arg-NA	25	5	73	81
pyro-Glu-Gly-Arg-NA	0	0	0	85
Bz-Ile-Glu-Gly-Arg-NA	0	0	0	100
Cbz-Val-Gly-Arg-NA	4	0	3	50
H-D-Val-Leu-Arg-NA	25	100	67	36
Tos-Gly-Pro-Lys-NA	4	0	5	46
H-D-Val-Leu-Lys-NA	0	0	8	12
Meo-Suc-Arg-Pro-Tyr-NA	0	0	0	0
Boc-Ala-Ala-NA	0	0	0	0

Purified TSP-1, t-kallikrein, p-kallikrein and pancreatic trypsin were tested against the substrates indicated. Amidolytic activity was determined as described in section 2

was separated on polyacrylamide gels and visualized by autoradiography. The polymerase activity of untreated RT_{MoMuLV} in the system is indicated by a labeled DNA band of about 600 bases as expected for rabbit globin mRNA (fig.2, lanes 1,7,12). Pretreatment of RT_{MoMuLV} with increasing concentrations of TSP-1 resulted in a dose-dependent reduction of polymerase activity (fig.2, lanes 2–5). Preincubation of TSP-1 with PMSF abolished this effect (fig.2, lane 6). Furthermore, enzyme activity of RT_{MoMuLV} was also considerably reduced after incubation with t-kallikrein (fig.3, lanes 10,11) and was totally abolished after treatment with pancreatic trypsin (fig.2, lanes 13,14) at enzyme concentrations comparable to that of TSP-1. In contrast, p-kallikrein reduced polymerase activity of RT_{MoMuLV} only marginally (fig.3, lanes 8,9). Inactivation of RT_{MoMuLV} by pancreatic trypsin was reversed to a great extent after pretreatment of the enzyme with PMSF (fig.2, lane 15). The differential capacity of t-kallikrein vs p-kallikrein to inactivate RT_{MoMuLV} is not unexpected; in spite of their similar amidolytic activities, as observed on model peptide substrates, the two enzymes recognize different cleavage sites on the protein substrate high-molecular-mass kininogen [28,29]. Furthermore, low-molecular-mass kininogen was shown to be only a substrate for t- but not for p-kallikrein [29]. Thus, the results demonstrate that TSP-1 is able to efficiently inactivate RT_{MoMuLV}. Moreover the finding that the polymerase is only partially inactivated by t-kallikrein and not at all by p-kallikrein stresses the highly restricted substrate specificity of individual proteinases on native proteins.

Table 2

Hydrolysis of the protein substrate azocoll by TSP-1, t-kallikrein and pancreatic trypsin

	Hydrolysis (A/h per mg enzyme)		
	TSP-1	t-Kallikrein	Trypsin
Azocoll	24	24	7600

Purified TSP-1, t-kallikrein and pancreatic trypsin were tested for proteolytic activity on azocoll as described in section 2

3.4. *Cleavage of RT by TSP-1*

In order to investigate whether the inactivation of RT_{MoMuLV} by TSP-1 and similar serine proteinases is accompanied by controlled proteolytic degradation, aliquots of RT_{MoMuLV} were incubated

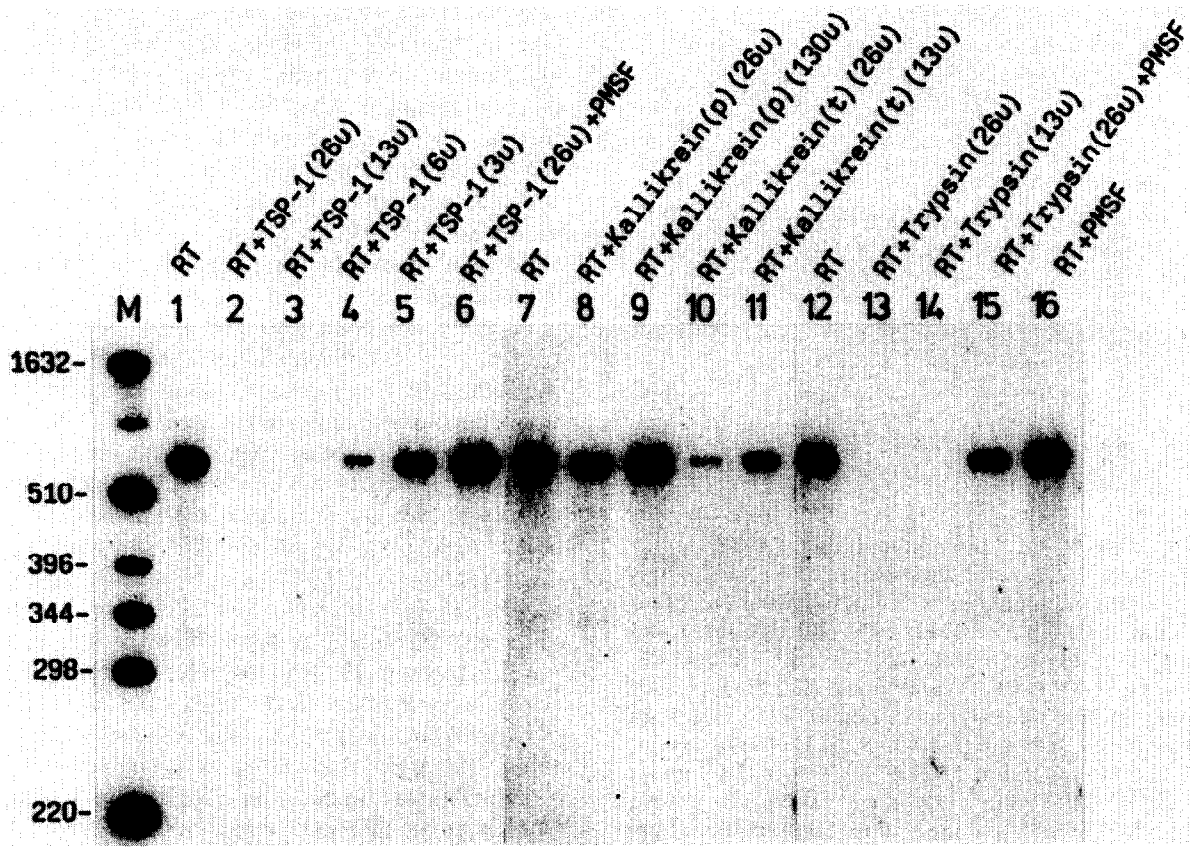

Fig.2. Effect of serine proteinase on the polymerase activity of RT_{MoMuLV}. Residual activity of RT_{MoMuLV} was determined after pretreatment with various concentrations of the following proteases: (1,7,12) without protease; (2) 26 U (208 ng) TSP-1; (3) 13 U (104 ng) TSP-1; (4) 6 U (52 ng) TSP-1; (5) 3 U (26 ng) TSP-1; (6) 26 U (208 ng) TSP-1 and PMSF (3 mM); (8) 26 U p-kallikrein; (9) 130 U p-kallikrein; (10) 26 U (32.5 ng) t-kallikrein; (11) 13 U (16 ng) t-kallikrein; (13) 26 U (49 ng) trypsin; (14) 13 U (25 ng) trypsin; (15) 26 U (49 ng) trypsin and PMSF (3 mM); (16) PMSF (3 mM) alone. The reverse transcriptase assay was performed as described in section 2. (M) Size-markers (pBR322 *Hin*fI fragments in bases) are shown to the left.

with purified preparations of TSP-1, kallikrein (t) or with pancreatic trypsin, previously adjusted to express similar amidolytic activity on PFR-NA. Subsequently, the samples were fractionated on SDS-PAGE under reducing conditions and analysed by protein staining. The serine proteinases on their own were either undetectable or showed up as very faint bands under these conditions. The preparation of the cloned RT_{MoMuLV} (>99% purity) showed a protein band of 80 kDa (fig.3, lane 10), in agreement with the molecular mass of the viral enzyme [31]. Treatment of RT_{MoMuLV} with TSP-1 resulted in the disappearance of the RT-specific band and the appearance of two major and one minor species with a molecular mass around 33, 18 and 31 kDa, respectively (fig.3, lane 1). Pretreatment of TSP-1 with either PMSF or PFR-CK abolished its proteolytic activity on RT_{MoMuLV} (fig.3, lanes 2,3). The inhibitors alone had no effect on the polymerase's integrity (fig.3, lanes 4,5) and also did not influence the activity of RT_{MoMuLV} (fig.2, lane 16). Furthermore, RT_{MoMuLV} was also partially cleaved by t-kallikrein (fig.3, lane 6). Moreover, only one minor band with a molecular

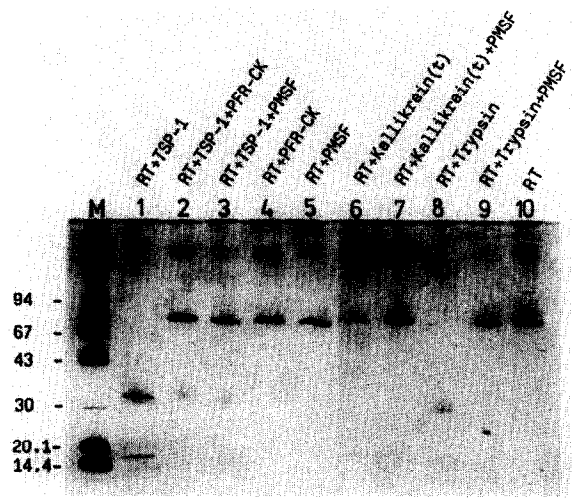

Fig.3. SDS-PAGE analysis (reducing conditions) of RT_{MoMuLV}. Proteolytic degradation of RT_{MoMuLV} was tested after pretreatment with the following proteases: (1) 26 U (208 ng) TSP-1; (2) 26 U (208 ng) TSP-1 and PFR-CK (1 mM); (3) 26 U (208 ng) TSP-1 and PMSF (3 mM); (4) PFR-CK (1 mM) alone; (5) PMSF (3 mM) alone; (6) 26 U (32.5 ng) t-kallikrein; (7) 26 U (32.5 ng) t-kallikrein and PMSF (3 mM); (8) 26 U (49 ng) trypsin; (9) 26 U (49 ng) trypsin and PMSF (3 mM); (10) without protease. SDS-PAGE analysis and staining of protein with silver nitrate was done as described in section 2. (M) Relative molecular mass markers (in thousands) are shown on the left.

mass of 30 kDa was seen after pretreatment of RT_{MoMuLV} with pancreatic trypsin (fig.3, lane 8) indicating a more pronounced degradation of RT_{MoMuLV}. Proteolytic activity of both serine proteinases, t-kallikrein and pancreatic trypsin, on RT_{MoMuLV} was reduced to a great extent following treatment with PMSF (fig.3, lanes 7,9).

4. DISCUSSION

This study demonstrates that RT_{MoMuLV} is a suitable substrate for the T cell-associated serine proteinase TSP-1 which destroys the enzyme activity of RT_{MoMuLV} via limited proteolysis resulting in only few distinct degradation products. Similar results were also obtained using a reverse transcriptase preparation derived from avian myeloblastosis virus (not shown). Since the deduced protein sequence of RT_{MoMuLV} does not contain the tripeptide sequence PFR per se [32] it is likely that several tripeptide sequences with the primary AA arginine and AA substituents in positions P_2 and P_3 quite similar to that of PFR like Phe-Pro-Arg (FPR), Pro-Tyr-Arg (PYR) and Pro-Trp-Arg (PWR) serve as target structures for TSP-1. One could also imagine that the AA sequence in the model peptide mimics a similar AA composition formed by three-dimensional folding in the native substrate [24].

Is there any significance of this finding with respect to T cell-mediated antiviral defense mechanisms? Experimental work in mouse and man suggests the importance of CTLs in the control of virus replication. The generation of CTLs has been demonstrated for both DNA and RNA viruses including retroviruses such as murine sarcoma virus [33], Friend [34,35], Rauscher [36], AKR/Gross LV [36,37] and MoMuLV [38]. In several in vivo studies it was shown that cloned virus-specific CTLs protect mice against lethal virus infections [2–4]. However, although these results were taken as evidence that the elimination of virus from the host was due to lysis of infected target cells there is no direct evidence yet for this T cell-effector function in vivo. More recently, Walker et al. [39] have demonstrated that $CD8^+$ lymphocytes derived from HIV antibody positive individuals were able to suppress HIV replication in selected $CD4^+$ cells in vitro at the initiation of retrovirus production as well as after several weeks of retrovirus replication by cultured $CD4^+$ cells. The data suggested that $CD8^+$ cells do not inhibit virus replication by suppression or lysis of HIV-infected $CD4^+$ cells but rather by an as yet undefined soluble mediator distinct from IFNγ. It has been shown recently that the CTL-target cell interaction is accompanied by directed granule exocytosis [40–42]. Thus, since TSP-1 is associated with cytoplasmic granules of CTL [10,12,15] and is specifically released by CTLs into the extracellular space upon contact with target cells [11,14,16], TSP-1 may interact with proteins from virus particles following CTL-target cell interaction. At present one can only speculate on how soluble TSP-1 could meet and react with its substrate RT. It is possible that the serine proteinase is bound to and endocytosed together with intact viruses resulting in accumulation of both entities in the cytoplasma of newly infected cells. Alternatively, TSP-1 may

be internalized during constitutive endocytosis events by infected cells. TSP-1 may also penetrate into host cells during the CTL-target cell interaction possibly via membrane channels formed by cytolysin/perforin molecules in target cell membranes [40–42]. All hypothetical processes could facilitate an encounter of TSP-1 with retroviral RT in appropriate compartments of the cytosol resulting in blockade of the key step in the reproductive cycle of retroviruses – copying the single-stranded RNA genome into double-stranded DNA [26] – by cleaving RT present in the virion after its uncoating.

However, it is also possible that proteins of the envelope of intact retroviruses are substrates for TSP-1. In this case, TSP-1 might interfere with the binding of virus particles to cell surface receptors by degrading anti-receptor determinants via limited proteolysis. The fact that the envelope protein of MoMuLV contains multiple AA tripeptide sequences similar to PFR has prompted us to initiate studies in this direction.

The message of this study is that CTLs express and secrete a serine proteinase which has the ability to cleave and inactivate the retroviral protein RT. Although the mechanism(s) of interference with retroviral replication by CTLs is (are) far from being resolved, the finding reported here offers a new approach to investigate the T cell-mediated control of retroviral infections.

ACKNOWLEDGEMENTS

We thank Ms G. Nerz and M. Prester for excellent technical assistance. The authors also thank Drs H. Eibel, K. Eichmann, G. Köhler, J. Langhorne, R. Lamers, G. McMaster, P. Nielson, and H.D. Wolf for critically reading this manuscript, Drs H. Eibel and R. Wallich for their help in screening protein sequence data banks, and Ms R. Brugger and R. Schneider for secretarial help. This work was in part supported by a grant from the Deutsche Forschungsgemeinschaft Si 214/5-4.

REFERENCES

[1] Zinkernagel, R.M. and Doherty, P.C. (1979) Adv. Immunol. 27, 52–177.
[2] Zinkernagel, R.M. and Welsh, R.M. (1976) J. Immunol. 117, 1495–1502.
[3] Yap, K.L., Ada, G.L. and McKenzie, I.F.C. (1978) Nature 273, 238–239.
[4] Lin, Y.L. and Askonas, B.A. (1981) J. Exp. Med. 154, 225–232.
[5] Prystonsky, M.B., Ely, J.M., Beller, D.I., Eisenberg, L., Goldman, J., Goldman, M., Goldwasser, E., Ihle, J., Quintans, J., Remald, H., Vogel, S.N. and Fitch, F.W. (1982) J. Immunol. 129, 2337–2344.
[6] Morris, A.G., Lin, Y.L. and Askonas, B.A. (1982) Nature 295, 150–152.
[7] Klein, J.R., Raulet, D.H., Pasternack, M.S. and Bevan, M.J. (1982) J. Exp. Med. 155, 1198–1203.
[8] Simon, M.M., Hoschützky, H., Fruth, U., Simon, H.-G. and Kramer, M.D. (1986) EMBO J. 5, 3267–3274.
[9] Kramer, M.D., Binninger, L., Schirrmacher, V., Moll, H., Prester, M., Nerz, G. and Simon, M.M. (1986) J. Immunol. 136, 4644–4651.
[10] Masson, D., Nabholz, M., Estrade, C. and Tschopp, J. (1986) EMBO J. 5, 1595–1600.
[11] Pasternack, M.S., Verret, C.R., Lin, M.A. and Eisen, H.N. (1986) Nature 322, 740–741.
[12] Young, J.D.E., Leong, L.G., Lin, C.-C., Dimiano, A., Wall, D.A. and Cohn, Z.A. (1986) Cell 47, 183–194.
[13] Simon, M.M., Fruth, U., Simon, H.-G. and Kramer, M.D. (1986) Eur. J. Immunol. 16, 1559–1568.
[14] Garcia-Sanz, J.A., Plaetnick, G., Velotti, F., Masson, D., Tschopp, J., MacDonald, H.R. and Nabholz, M. (1987) EMBO J. 6, 933–938.
[15] Fruth, U., Prester, M., Golecki, J.R., Hengartner, H., Simon, H.-G., Kramer, M.D. and Simon, M.M. (1987) Eur. J. Immunol. 17, 613–621.
[16] Simon, M.M., Fruth, U., Simon, H.-G. and Kramer, M.D. (1987) Ann. Inst. Pasteur 138, 309–314.
[17] Simon, M.M., Simon, H.-G., Fruth, U., Epplen, J.T., Müller-Hermelink, H.K. and Kramer, M.D. (1987) Immunology 60, 219–230.
[18] Kramer, M.D. and Simon, M.M. (1987) Immunol. Today 8, 140–142.
[19] Simon, M.M., Weltzien, H.U., Bühring, H.J. and Eichmann, K. (1984) Nature 308, 367–370.
[20] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[21] Laemmli, U.K. (1970) Nature 227, 680–685.
[22] Chavira, R., Burnett, T.J. and Hagemann, J.H. (1984) Anal. Biochem. 136, 446–450.
[23] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

[24] Cleason, G., Annell, L., Karlsson, G., Gustavsson, S., Friberger, P., Avielly, S. and Simonsson, R. (1977) in: New Methods Further Analysis of Coagulation Using Chromogenic Substrates (Witt, I. ed.) pp.37–54, Walter de Gruyter, Berlin.
[25] Achstetter, T. and Wolf, D.H. (1985) EMBO J. 4, 173–177.
[26] Temin, H.M. and Baltimore, D. (1972) Adv. Virus Res. 17, 129–186.
[27] Kranz, D.M., Pasternak, M.S. and Eisen, H.N. (1987) Fed. Proc. 46, 309–312.
[28] Kato, H., Nagasawa, S. and Iwanagar, S. (1981) Methods Enzymol. 80, 172–198.
[29] Iwanage, S., Kato, H., Sugo, T., Ikari, N., Hashimoto, N. and Fujii, S. (1979) Biological Functions of Proteinases (Holzer, H. and Tschesche, H. eds) pp.243–259, Springer, Berlin.
[30] Barret, A.J. and Salvesen, G. (1986) Proteinase Inhibitors, Elsevier, Amsterdam, New York.
[31] Gerard, G.F. (1981) Biochemistry 20, 256–265.
[32] Shinnick, M., Lerner, R.A. and Sutcliffe, J.G. (1981) Nature 293, 543–548.
[33] Gomard, E., Duprez, V., Henin, Y. and Levy, J.P. (1976) Nature 260, 707–709.
[34] Chesebro, B. and Wehrly, K.J. (1976) Exp. Med. 143, 85–99.
[35] Blank, K.J., Freedman, H.A. and Lilly, F. (1976) Nature 260, 250–252.
[36] Plata, F. and Lilly, F.J. (1979) Exp. Med. 150, 1174–1186.
[37] Green, W.R. (1980) J. Immunol. 125, 2584–2590.
[38] Flyer, D.C., Burakoff, S.J. and Faller, D.V. (1986) J. Immunol. 137, 3968–3972.
[39] Walker, C.M., Moody, D.J., Stites, D.P. and Levy, J.A. (1986) Science 234, 1563–1566.
[40] Henkart, P.A. (1985) Annu. Rev. Immunol. 3, 31–58.
[41] Podack, E.R. (1985) Immunol. Today 6, 21–27.
[42] Young, J.D.E. and Cohn, Z.A. (1986) Cell 46, 641–642.