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Total chemical synthesis of human T-cell leukemia virus type 1 (HTLV-1) protease via native chemical ligation:

Reexamination of the functional effect of C-terminal truncation

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Abstract

Human T-cell leukemia virus 1 (HTLV-1) protease - a member of the aspartic acid protease family plays critical roles in the pathogenesis of the virus and is an attractive viral target for therapeutic intervention. HTLV-1 protease consists of 125 amino acid residues and functions as a homodimer stabilized in part by a four-stranded β -sheet comprising the N- and C-termini. Compared with many other viral proteases such as HIV-1 protease, HTLV-1 protease is elongated by an extra 10 amino acid residue "tail" at the C-terminus. The structural and functional role of the extra C-terminal residues in the catalysis of HTLV-1 protease has been a subject of debate for years. Using the native chemical ligation technique pioneered by Kent and colleagues, we chemically synthesized a full-length HTLV protease and a C-terminally truncated form encompassing residues 1–116. Enzyme kinetic analysis using three different peptide substrates indicated that truncation of the C-terminal tail lowered the turnover number of the viral enzyme by a factor of 2 and its catalytic efficiency by roughly tenfold. Our findings differ from the two extreme views that the C-terminal tail of HTLV-1 protease is either fully dispensable or totally required for enzyme dimerization and/or catalysis.

Human T-cell leukemia virus type 1 (HTLV-1) – the first human retrovirus discovered by Gallo and colleagues in the late 1970s,¹ is clinically associated with adult T-cell leukemia, tropical spastic paraparesis or HTLV-1 associated myelopathy, and several other chronic diseases.^{2–5} It is estimated that 20–30 million of the world population is infected with HTLV-1, and 5–10% of these infected individuals will develop HTLV-1 associated diseases.^{6,7} No effective treatment is currently available for HTLV-1 infection, making the virus a dangerous emerging pathogen as classified by CDC.

Virally encoded proteases (PR) play an essential role in the life cycle of all retroviruses as viral assembly, maturation and replication necessitate proteolytic cleavage of Gag and Gag-Pro-Pol polyproteins into structural (matrix, capsid, and nucleocapsid) as well as functional (reverse transcriptase and integrase) proteins.⁸ This, along with the successful development of HIV-1 PR inhibitors for the treatment of AIDS, validates HTLV-1 PR as an attractive viral target for therapeutic intervention in HTLV-1 infection.

HTLV-1 PR of 125 amino acid residues and HIV-1 PR of 99 amino acid residues are members of the aspartic acid protease family, and function as a structurally conserved homodimer.⁹ Aside from their significantly different substrate specificity and inhibition profile due to relatively low sequence identity,¹⁰ the two viral proteases differ in the C-terminal region. HTLV-1 PR C-terminally is elongated by 10 amino acid residues

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(P¹¹⁶EAKGPPVIL¹²⁵) as compared with HIV-1 PR – an attribute shared only by leukemia virus proteases.¹¹

Hayakawa et al. first reported in 1992 that while the very last five residues (P¹²¹PVIL¹²⁵) were functionally dispensable, residues 116–120 (P¹¹⁶EAKG¹²⁰) were required for the auto-processing activity of HTLV-1 PR.¹² However, sequence alignment, structure modeling, and functional characterizations of truncation mutants of HTLV-1 PR suggest that the extra C-terminal residues exist as a flexible tail distal to the active site of the enzyme, thus playing limited structural or functional roles.^{10,12,13} This tenet recently received support from structural studies by Wlodawer and colleagues of a truncated HTLV-1 PR (1–116), which retained 60% of the catalytic activity of its full-length counterpart.¹⁴ Nevertheless, the controversy still remains as evidenced by a latest report in 2008 that deletion of residues 116–125 or 117–125 in HTLV-1 PR rendered the protease completely inactive.¹⁵ The authors attributed the lack of activity of HTLV-1 PR (1–115) or HTLV-1 PR (1–116) to its inability to dimerize.

These conflicting findings triggered us to carry out comparative functional studies of a full-length protease and its C-terminally truncated form HTLV-1 PR (1–116), both of which were chemically synthesized using the native chemical ligation (NCL) technique pioneered by Kent and colleagues.¹⁶ Stepwise chemical syntheses of active bovine leukemia virus PR (1–126) and (1–116) were reported in 1992 and 1993,^{17,18} followed by the synthesis of HTLV-1 PR (1–125) first in 1997,¹⁹ and then in 2002.²⁰ However, no comparative activity data were generated for those synthetic enzymes to allow the delineation of the functional effect of C-terminal truncation. Using three different peptide substrates, we demonstrated in this report that truncation of the extra C-terminal residues of HTLV-1 PR reduced the catalytic efficiency of the viral protease by tenfold and lowered its turnover number by twofold.

MATERIALS AND METHODS

Materials

Boc-(L)-amino acids and Boc-(D)-Ser(Bzl)-OH were purchased from Peptides International (Louisville, KY); Boc-Pro-OCH₂-Pam (4-(hydroxymethyl)-phenylacetamidomethyl)-, Boc-Ser(Bzl)-OCH₂-Pam, and Boc-Lys(Cl-Z)-OCH₂-Pam resins were obtained from Applied Biosystems (Foster City, CA); dichloromethane, N,N-dimethylformamide, and HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA), and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU)¹ was purchased from American Bioanalytical (Natick, MA). Trifluoroacetic acid (TFA) was acquired from Halocarbon (River Edge, NJ) and hydrogen fluoride (HF) from Matheson Tri-gas (Montgomeryville, PA). N,N-Diisopropylethylamine (DIEA), thiophenol, and *p*-cresol were from Fluka (Switzerland), and ultrapure guanidinium hydrochloride was from ICN Biochemicals (Irvine, CA).

Solid phase peptide synthesis

The amino acid sequence of the 125-residue HTLV-1 PR is shown below:

PVIPLDPARR¹⁰ PVKAQVDTQ²⁰ TSHPKTIEAL³⁰ LDTGADMTVL⁴⁰ PIALFSSNTP⁵⁰
LKNTSVLGAG⁶⁰ GQTQDHF⁷⁰SLPVLIRLPF⁸⁰ RTTPIVLTSC⁹⁰ LVDTKNNPAI¹⁰⁰
IGRDALQQCQ¹¹⁰ GVLYLPEAKR¹²⁰ PPVIL¹²⁵

¹(Abbreviations: HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate,; TFA, trifluoroacetic acid; HF, hydrogen fluoride; DIEA, N,N-diisopropylethylamine; RP-HPLC, reversed-phase HPLC; ESI-MS, electrospray ionization mass spectrometry.)

Our synthetic constructs contained an S47C mutation for NCL and an L40I mutation that was reported to suppress known autolysis of the protease without altering its catalytic activity.²¹ In addition, the full-length PR contained an extra Pro¹²⁶ residue at the C-terminus. Four peptide segments of HTLV-1 PR, i.e., H-(1–46) α COSR, H-(47–89) α COSR, H-(90–126)-OH and H-(90–116)-OH were individually synthesized on appropriate resins on an ABI 433A peptide synthesizer using an optimized HBTU activation/DIEA *in situ* neutralization protocol developed by Kent and coworkers for Boc chemistry SPPS.²² The same chemistry was used to prepare the thioester-generating resin (Boc-Ser(Bzl)- α COSCH₂CO-Leu-OCH₂-PAM resin) by coupling Boc-Ser(Bzl)-OH to custom-made HSCH₂CO-Leu-OCH₂-PAM-resin. Cys⁴⁷ was orthogonally protected by acetamidomethyl (Acm). Three oligopeptide substrates derived from the proteolytic processing sites of HTLV-1 Gag polyprotein, i.e. MA/CA (APQVL PVMHP), CA/NC (KTKVL VVQPK) and MA/nitroCA (APQVL^{nitro} FVMHP),²³ as well as the two N-terminal hydrolysis products (APQVL and KTKVL) were also prepared. After chain assembly, side chain protecting groups were removed and peptides cleaved from the resin by treatment with anhydrous HF and *p*-cresol (9:1) at 0 °C for 1 h. Crude peptides were precipitated with cold ether and purified by preparative C₁₈ reversed-phase (RP) HPLC to homogeneity, and their molecular masses ascertained by electrospray ionization mass spectrometry (ESI-MS).

Native chemical ligation

Native chemical ligation between H-(47–89) α COSR and H-(90–126)-OH or H-(90–116)-OH was carried out in 0.1 M phosphate buffer containing 6 M guanidine hydrochloride and 2% (v/v) thiophenol, pH 7.4. The ligation reaction went to completion overnight, as judged by HPLC and ESI-MS analyses, and the ligation products (H-(47–126)-OH and H-(47–116)-OH) were chromatographically purified. Deprotection of Cys⁴⁷(Acm) was achieved by dissolving the ligation product, at 1 mg/ml, in 50% acetonitrile containing 0.1% TFA, to which 300-fold molar excess of silver acetate (at 5 mg/ml) was added. The reaction proceeded for 1 h before being quenched by DTT. After HPLC purification, H-(47–126)-OH or H-(47–116)-OH was subsequently ligated to (1–46) α COSR essentially as described, giving rise to HTLV-1 PR (1–126) and HTLV-1 PR (1–116).

Protein folding

Folding of HTLV-1 PR (1–126) and HTLV-1 PR (1–116) was achieved using a published protocol with modification.²⁴ Briefly, HTLV-1 PR (1–126) or HTLV-1 PR (1–116) was dialyzed first against 25 mM formic acid, pH 2.8, and subsequently against 50 mM sodium acetate buffer containing 1 mM DTT and 1 mM EDTA, pH 5.0, followed by a final dialysis against 20 mM PIPES buffer containing 150 mM NaCl, 10% glycerol, 1 mM EDTA, 2 mM DTT, and 0.5% Nonidet P-40, pH 7.0. Protein solutions were quantified by UV measurements at 280 nm using a molar extinction coefficient of 1490 M⁻¹cm⁻¹ calculated according to a published algorithm.²⁵

Enzyme kinetics

The PR activity assay was essentially as described.²⁴ Specifically, 10 μ L of a freshly folded protease was diluted into 50 μ L of a 2 $\times$ assay buffer (0.5 M phosphate, 10% glycerol, 2 mM EDTA, 10 mM DTT, 4 M NaCl, pH 5.6), to which 40 μ L of peptide substrate was added. The final concentration of peptide substrate varied from 0.125 to 8 mM, depending on the K_m values. After an incubation at 37 °C for 1 h, the reaction was terminated by adding 50 μ L 1% TFA. An aliquot of 75 μ L reaction mixture was injected onto a Waters Symmetry 300 C₁₈ column (4.6 $\times$ 50 mm, 5 μ m) running a 30-min linear gradient of 5–65% acetonitrile containing 0.1% TFA at a flow rate of 1 mL/min at 40 °C. Cleavage products were identified by ESI-MS, and quantified using calibration curves pre-established by HPLC analysis of APQVL and KTKVL at different concentrations. The experimental data were

fitted to the Michaelis-Menten equation using Graphpad Prism 4 (GraphPad Software, Inc.), yielding kinetic parameters, k_{cat} and K_m , for HTLV-1 PR (1–126) and HTLV-1 PR (1–116).

RESULTS

Protein synthesis via native chemical ligation

The strategy for the synthesis of HTLV-1 PR is illustrated in Figure 1. We mutated Ser47 to Cys in order to produce the target polypeptide using three-segment native chemical ligation. Since the mutation site is located in a loop region distal to the active site of the enzyme, minimal structural and functional impact is expected. The 1st ligation between H-(90–126)-OH and H-(47–89) α COSR yielded a readily separable intermediate product H-(47–126)-OH. To prevent an intramolecular ligation (head-to-tail backbone cyclization) of H-(47–89) α COSR, Cys⁴⁷ was orthogonally protected by Ac_m, which required deprotection prior to the 2nd ligation reaction between (1–46) α COSR and H-(47–126)-OH. Methods for Ac_m removal are well documented in the literature,^{26–28} often involving treatment of Cys(Ac_m)-containing peptides with iodine or heavy metal ions such as Ag⁺ and Hg²⁺. Silver acetate was used in this work to quantitatively remove Ac_m, giving rise to an almost 70% yield of ligation product with a free N-terminal Cys residue after HPLC purification.

The 2nd ligation reaction was also quantitative, yielding the full-length HTLV-1 PR whose molecular mass was determined to be 13672.1 ± 0.9 Da, within experimental error of the theoretical value of 13672.1 Da calculated on the basis of the average isotopic compositions of HTLV-1 PR (1–126) (Figure 2A). Synthesis of HTLV-1 PR (1–116) was essentially the same as described above, except that H-(90–116)-OH was used as the C-terminal fragment. The determined molecular mass of HTLV-1 PR (1–116) was 12570.4 ± 0.6 Da, in agreement with the expected value of 12570.7 Da (Figure 2B). On a 0.25 mmol scale of synthesis, ~ 65 mg of highly purified HTLV-1 PR (1–126) and 70 mg of pure HTLV-1 PR (1–116) were obtained.

Folding of HTLV-1 PR (1–126) and HTLV-1 PR (1–116)

Different folding procedures have been described in the literature for HTLV-1 PR. Herger et al. directly dialyzed a recombinant HTLV-1 PR and its deletion mutant against 10 mM sodium acetate, pH 5.3, at 4 °C.¹¹ Ding et al. sequentially dialyzed recombinant HTLV-1 PR first against 10 mM sodium acetate, pH 3.5 and, then, against 100 mM sodium citrate containing 5 mM EDTA, 1 mM DTT, and 1 M NaCl, pH 5.3.²³ Kadas et al. developed a different and “seemingly cumbersome” dialysis protocol,²⁴ as detailed in the Materials and Methods section. We tried all three different protocols for the folding of synthetic HTLV-1 PR, and found that the yield and catalytic efficiency of the “folded” enzyme(s) were strongly protocol-dependent. The Kadas protocol resulted in the highest recovery and enzymatic activity, whereas the Herger protocol fared worst (data not shown).

Enzymatic activity assays

The enzymatic activity of the full-length HTLV-1 PR and HTLV-1 PR (1–116) was determined in a kinetic assay that measured on RP-HPLC the rate of hydrolysis of the three oligopeptide substrates, MA/CA, CA/NC, and MA/^{nitro}CA. To minimize auto-proteolysis of HTLV-1 PR,^{29,30} freshly folded enzymes were employed throughout the assay. Both forms of synthetic HTLV-1 PR specifically cleaved all three peptide substrates at the expected maturation sites –LP-, –LV-, or –L^{nitro}F-, releasing the N-terminal product APQVL (from MA/CA and MA/^{nitro}CA) or KTKVL (from CA/NC). Shown in Figure 3 is a representative cleavage reaction for HTLV-1 and MA/^{nitro}CA at different enzyme/substrate ratios, monitored on RP-HPLC and verified by ESI-MS.

Kinetic data were fitted to the Michaelis-Menten equation, yielding k_{cat} and K_{m} values for the two enzymes and three substrates (Figure 4). For the two natural substrates MA/CA and CA/NC, the turnover number k_{cat} of the full-length HTLV-1 PR was only marginally higher than that of HTLV-1 PR (1–116). Due to a greater disparity in K_{m} , however, the catalytic efficiency (as measured by the $k_{\text{cat}}/K_{\text{m}}$ ratio) of the full-length protease was 7–9 fold better than the truncated form. Similar results were obtained with the unnatural substrate MA/^{nitro}CA, where HTLV-1 PR (1–126) was 10 fold more efficient than HTLV-1 PR (1–116). These data indicate that truncation of the C-terminal residues in HTLV-1 PR, while causing a notable reduction in protease activity, does not negate it, contrasting the previously published results by Kadas et al.^{11,15}

Possible racemization at the Ser⁸⁹-Cys⁹⁰ ligation site

Interestingly, ligation of H-(47–89) α COSR to H-(90–126)-OH resulted in two chromatographically distinct products at a ratio of 1:4 (Figure 5A). Their identical masses determined ruled out a deamidation reaction; their resistance to base treatment eliminated the possibility of formation of a β -ester linkage in H-(47–89) α COSR that can potentially result from an N-to-O acyl shift rearrangement at a Ser residue during HF cleavage.³¹ We suspected that a possible racemization reaction occurred at the Ser⁸⁹-Cys⁹⁰ ligation site during transthioesterification, resulting in a minor product with a shorter retention time. Racemization during NCL is generally rare, and only slight racemization at the His–Cys, Leu–Cys and Phe–Cys ligation sites has been documented.^{32,33}

To investigate the possibility of Ser⁸⁹ racemization, we examined the ligation reaction of two model peptides derived from HTLV-1 PR, i.e., H-(84–89) α COSR (PIVLTS α COSR) and H-(90–95)-OH (CLVDTK). Two diastereomers of the N-terminal thioester-bearing peptide were synthesized – one containing an L-Ser⁸⁹, and the other a D-Ser⁸⁹. As chromatographic controls, the ligation products (PIVLTL-SCLVDTK and PIVLTD-SCLVDTK) were also prepared using stepwise synthesis. As shown in Figures 5B and 5C, each ligation reaction resulted in a minor product with a retention time different from and a molecular mass identical to that of the major product. HPLC analysis of the chromatographic controls suggested that a racemization at Ser⁸⁹ likely occurred during the ligation reaction. Notably, racemization appeared significantly less severe with the short model peptides than with their longer parent peptides, indicative of a context (sequence) – dependent chemical event. There was no evidence of racemization at the second ligation site (Ser⁴⁶-Cys⁴⁷). Further experimental work needs to be undertaken to investigate this apparent racemization during native chemical ligation.

To evaluate the effect of Ser⁸⁹ racemization on the enzymatic activity of HTLV-1 PR, we prepared (L-S⁸⁹→D-S⁸⁹)-HTLV-1 PR (1–126) and quantified its ability to hydrolyze the CA/NC substrate (Figure 5D). Compared with HTLV-1 PR (1–126), the diastereomeric protease showed a decreased k_{cat} of 2.47 s^{–1} and an increased K_{m} of 1.92 mM. Overall, the data suggest that Ser⁸⁹ racemization reduced the catalytic efficiency ($k_{\text{cat}}/K_{\text{m}}$) of the enzyme by tenfold – on par with the deleterious effect of the C-terminal truncation.

Discussion

Native chemical ligation is a revolutionary synthetic methodology pioneered by Kent and coworkers that enables two or more fully unprotected peptides to chemo-selectively react in aqueous solution, forming a longer polypeptide with high efficiency and yield.^{16,34} Many proteins have been chemically synthesized using the NCL technique,³⁵ including enzymes such as HIV-1 PR,³⁶ ribonuclease A,³⁷ and lysozyme,³⁸ significantly advancing our understanding of the molecular basis of protein function in a way that was previously unattainable. A number of varied forms of NCL have also been established,^{39–43} further

expanding the capacity and augmenting the power of this technology. The findings reported here illustrate the use of NCL for tackling a controversial issue regarding the functional importance of the extra C-terminal residues in HTLV-1 PR.

In HIV-1 PR and HTLV-1 PR, the N- and C-termini form an interspersed, four-stranded anti-parallel β -sheet structure important for protease dimerization and activity.⁴⁴

Although the crystal structure of a full-length HTLV-1 PR is not available, the crystal structure of the C-terminally truncated form HTLV-1 PR (1–116) suggested that the extra C-terminal residues did not directly participate in the β -sheet formation, and had a limited structural impact on protease dimerization.¹⁴ In fact, the catalytic activity of the crystallized HTLV-1 PR (1–116) was only marginally less active than a full-length protease, in agreement with several published reports based on sequence analysis, structural modeling and functional studies.^{10·12·13} Our observation that deletion of the extra C-terminal residues lowered the turnover number of HTLV-1 PR by a factor of 2 and its catalytic efficiency roughly by tenfold appears to support, to a large extent, these previous findings.

In sharp contrast, Kadas et al. reported that the extra 9 or 10 residues at the C-terminus of HTLV-1 PR were functionally indispensable.¹⁵ Based on the crystal structure of HTLV-1 PR (1–116), a modeling study by the authors suggested that the extra C-terminal residues could potentially form two additional β -strands to help stabilize the homodimer.¹⁵ Lack of the two additional β -strands would promote dimer dissociation as suggested by gel filtration and pull-down protein-protein interaction assays.¹⁵ Our results support a limited role the extra C-terminal residues play in HTLV-1 PR dimerization and catalysis. In our work their deletion is clearly insufficient to decimate protease dimerization or abolish its catalytic activity.

While these contradictory findings with respect to the functional importance of the extra C-terminal residues in HTLV-1 PR remain to be reconciled, it is worth pointing out that the literature is plagued by inconsistent functional assays of the protease itself. The reported catalytic activity of full-length HTLV-1 PR varies from group to group by as much as four orders of magnitude.^{11·21·23} For example, Kadas et al. reported a k_{cat}/K_m value of 158.7 $\text{mM}^{-1}\text{s}^{-1}$ for HTLV-1 PR and CA/NC, whereas Ding et al. obtained a k_{cat}/K_m ratio of 0.019 $\text{mM}^{-1}\text{s}^{-1}$ for the same enzyme and substrate.^{15·23} This huge discrepancy in k_{cat}/K_m is probably not totally unexpected in light of our observation that the enzymatic activity of synthetic HTLV-1 PR is strongly dependent on folding conditions. Autolysis, assay conditions, and sequence differences in the various HTLV-1 PR constructs may also be important contributing factors responsible for the documented discrepancies in the catalytic activity of the viral protease.

An interesting finding was made in the chemical synthesis of HTLV-1 PR using the NCL technique, implicating possible racemization of Ser⁸⁹. Ser⁸⁹ is located in a loop connecting two short β -strands (Figure 1), thus Ser⁸⁹ racemization could be structurally tolerated. As the loop is in close proximity to the active site of the enzyme, the deleterious effect of Ser⁸⁹ racemization is not unexpected. However, Ser⁸⁹-dependent racemization was relatively minor and the unracemized ligation product was readily purified, and large amounts of highly pure HTLV-1 PR were readily obtained, enabling future structural and functional studies of this important viral enzyme.

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Figure 1.

Strategy for the synthesis of HTLV-1 PR using three-segment native chemical ligation. The image of dimeric HTLV-1 PR was created by PyMol (DeLano Scientific LLC, <http://pymol.org>) using the coordinates for a truncated recombinant form (1–116) (PDB code: 2B7F) 14. The two ligation sites (site 1: Ser⁸⁹-Cys⁹⁰; site 2: Ser⁴⁶-Cys⁴⁷) and the catalytic residue Asp32 are highlighted in stick representation.

Figure 2.

Characterization of synthetic HTLV-1 PR (1–126) and HTLV-1 PR (1–116) by analytical HPLC and ESI-MS. The chromatograms were obtained on a Waters symmetry 300 C₁₈ column (4.6×150 mm, 5 μM) running a 30-min gradient of 5%–65% acetonitrile containing 0.1% TFA at a flow rate of 1 mL/min at 40 °C.

Figure 3. Cleavage of MA/nitroCA by HTLV-1 PR (1–126) at different enzyme/substrate ratios. **(A)** [Enzyme] = 850 nM; [substrate] = 300 μ M. **(B)** [Enzyme] = 1.25 μ M; [substrate] = 200 μ M. The cleavage reaction proceeded at 37 °C (in 250 mM phosphate, 5% glycerol, 1 mM EDTA, 5 mM DTT, 2 M NaCl, pH 5.6) for 1 h before being terminated by TFA acidification.

Figure 4.

Enzyme kinetic measurements of the catalytic activity of HTLV-1 PR (1–126) (left column) and HTLV PR (1–116) (right column) with the substrates MA/CA (**A**, **B**), MA/nitroCA (**C**, **D**), and CA/NC (**E**, **F**). The curves are averages of two independent measurements.

Figure 5.

Apparent racemization at Ser⁸⁹. (A) Ligation of H-(47-89)αCOSR to H-(90-126)-OH. The chromatogram was obtained at a linear gradient of 20–50% acetonitrile containing 0.1% TFA under otherwise the same conditions as described in the legend of Figure 2. (B) Ligation of PIVLT^LSαCOSR to CLVDTK. (C) Ligation of PIVLT^DSαCOSR to CLVDTK. The ligation reactions of the model peptides were monitored at 40 °C on a Waters Xbridge™ C₁₈ column (3.5 μm, 4.6 × 150 mm) running a 30-min linear gradient of 5–65% acetonitrile containing 0.1% TFA at a flow rate of 1 ml/min. (D) Enzyme kinetic measurements of the catalytic activity of (^LS89→^DS89)-HTLV-1 PR (1–126) with the substrate CA/NC. The curves are averages of two independent measurements.

Table 1

Kinetic parameters of HTLV-1 PR (1-126), HTLV-1 PR (1-116), and ^DS89 HTLV-1 PR (1-126) with three substrates, MA/CA, MA/^{micro}CA, and CA/NC.

Substrates	HTLV-1 PR (1-126)			HTLV-1 PR (1-116)			^D S89-HTLV-1 PR (1-126)		
	k_{cat} s ⁻¹	K_m mM	k_{cat}/K_m mM ⁻¹ s ⁻¹	k_{cat} s ⁻¹	K_m mM	k_{cat}/K_m mM ⁻¹ s ⁻¹	k_{cat} s ⁻¹	K_m mM	k_{cat}/K_m mM ⁻¹ s ⁻¹
MA/CA	8.31	0.80	10.4	4.28	3.75	1.14	n.d.	n.d.	n.d.
MA/ ^{micro} CA	0.161	0.042	3.82	0.0228	0.0618	0.37	n.d.	n.d.	n.d.
CA/NC	6.70	0.52	12.8	2.84	1.52	1.87	2.47	1.92	1.28

N.D. – not determined.