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Auto-inactivation by Cleavage within the Dimer Interface of Kaposi's Sarcoma-associated Herpesvirus Protease

Todd R. Pray[1], Anson M. Nomura[2], Michael W. Pennington[4] and Charles S. Craik[2,3]*

[1]*Graduate Group in Biophysics*

[2]*Department of Pharmaceutical Chemistry*

[3]*Departments of Biochemistry and Biophysics and Cellular and Molecular Pharmacology University of California San Francisco CA 94143-0446, USA*

[4]*BACHEM Biosciences, Inc. King of Prussia PA 19406, USA*

**Corresponding author*

An autolysis site of functional and structural significance has been mapped within the dimer interface of Kaposi's sarcoma-associated herpesvirus protease. Cleavage 27 residues from the C terminus of the 230 amino acid residue, 25 kDa protein was observed to cause a loss of dimerization and proteolytic activity, even though no active site moieties were lost. Gel-filtration chromatography and analytical ultracentrifugation were used to analyze the changes in oligomerization upon autolysis. The selective auto-disruption of this essential protein-protein interface by proteolytic cleavage resulted in a 60% loss in mean residue ellipticity by circular dichroism as well as a 20% weaker, 10 nm red-shifted intrinsic protein fluorescence emission spectrum. These apparent conformational changes induced a strict inhibition of enzymatic activity. An engineered substitution at the P1′ position of this cleavage site attenuated autolysis by the enzyme and restored wild-type dimerization. In addition to retaining full proteolytic activity in a continuous fluorescence-based enzyme assay, this protease variant allowed the determination of the enzyme's dimerization dissociation constant of 1.7 (±0.9) μM. The structural perturbations observed in this enzyme may play a role in viral maturation, and offer general insight into the allosteric relationship between the dimer interface and active site of herpesviral proteases. The functional coupling between oligomerization and activity presented here may allow for a better understanding of such phenomena, and the design of an enzyme variant stabilized to autolysis should further the structural and mechanistic characterization of this viral protease.

Keywords: conformational change; viral protease; spectroscopy; analytical ultracentrifugation; protein-protein interactions

Abbreviations used: KSHV (Pr), Kaposi's sarcoma-associated herpesvirus (protease); HSV, Herpes simplex virus; hCMV, human cytomegalovirus; VZV, varicella-zoster virus; MHV68, murine herpesvirus-68; EBV, Epstein-Barr virus; HVS, herpesvirus saimiri; EHV, equine herpesvirus; AHV, avian herpesvirus; sCMV, simian cytomegalovirus; HHV-6, human herpesvirus 6; M-site, maturation site; R-site, release site; I-site, internal site; D-site, dimer disruption site; CD, circular dichroism; ESI-MS, electrospray ionization mass spectrometry; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid; ORF, open reading frame.

E-mail address of the corresponding author: craik@cgl.ucsf.edu

Kaposi's sarcoma, the most common neoplasm affecting patients with acquired immunodeficiency syndrome, is linked to the presence of the recently identified Kaposi's sarcoma-associated herpesvirus (KSHV; reviewed by Ganem, 1997). As is the case for herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (hCMV), KSHV most likely requires a serine protease, KSHV Pr, encoded in its own genome for capsid assembly. The development of mature capsids by cleavage at the maturation site (M-site) and release site (R-site) of the virally encoded protease-assembly protein precursor molecule is contingent upon protease activity (reviewed by Gibson, 1996; Roizmann & Sears, 1996). KSHV Pr

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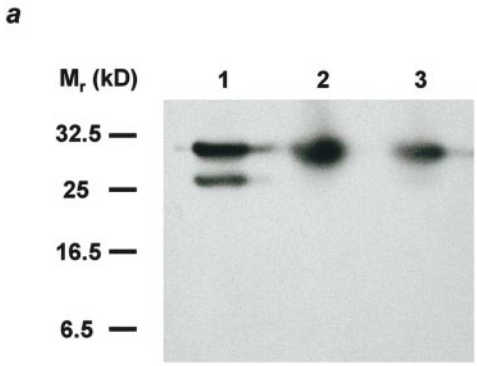
was sequenced, cloned, expressed and characterized preliminarily by our laboratory (Ünal *et al.*, 1997).

Significant biochemical and structural evidence suggests that dimerization is linked to enzymatic activity for HSV-1 and hCMV Pr. K_d values ranging from 0.55 to 50 μM have been determined through the use of light-scattering and chemical crosslinking (Margosiak *et al.*, 1996), centrifugation (Cole, 1996), and gel-filtration (Darke *et al.*, 1996; Schmidt & Darke, 1997). The three-dimensional structures of the proteases encoded by hCMV (Chen *et al.*, 1996; Qiu *et al.*, 1996; Shieh *et al.*, 1996; Tong *et al.*, 1996), HSV-1 and 2 (Hoog *et al.*, 1997), and varicella-zoster virus (VZV; Qiu *et al.*, 1997) reveal a novel fold, a Ser-His-His catalytic triad, and indicate that these proteins homodimerize through the association of α-helices near their C termini.

Although it is believed that dimerization is required for activity in this class of enzymes, neither the structural nor functional consequences of a loss of dimerization have been documented. Here, we find a direct correlation between loss of dimerization through autolysis at a naturally occurring site within KSHV Pr's dimer interface α-helix, and the subsequent loss of its proteolytic activity. Perturbation of the enzyme's secondary and tertiary structures, detected spectroscopically, accompanies this auto-inactivation and provides information regarding the possible regulatory role of dimerization for this protease.

Identification of an auto-inactivation site within KSHV protease

To facilitate the structural characterization of KSHV Pr, an expression system was developed to produce the enzyme in a soluble form. The KSHV Pr open reading frame (ORF) was amplified by PCR from the plasmid pBSλ21-5.8 (Ünal *et al.*, 1997), inserting a stop codon after Ala230 of the R-site, corresponding to Ala256 of hCMV Pr, and was ligated into the *Nco*I and *Hin*dIII sites of the vector pQE60 (Qiagen, Inc.) such that no His-tag was incorporated into the coding sequence of the enzyme. Initial studies demonstrated high expression levels in *Escherichia coli*, but indicated that a significant fraction of the protein was present as a lower molecular mass species (Figure 1(a)). Preliminary characterization indicated that the protease displayed hydrolytic activity against synthetic substrates. However, the loss of such activity within 60 minutes at high protease concentrations, and during the purification itself, was accompanied by further production of the lower molecular mass proteolytic fragment (data not shown). KSHV Pr S114A lacking the active-site serine (hCMV Pr residue 132), created by PCR-mediated site-directed mutagenesis, did not exhibit the same proteolytic pattern when expressed and harvested under identical conditions (Figure 1(a)). Thus, the cleavage of KSHV Pr most likely proceeded in an autolytic fashion and was not due to digestion by bacterial enzymes.



b

Virus	KSHV Residue Number 200	201	202	203	*	204	205	206	207	208
KSHV	A	I	D	A	*	S	F	I	R	D
MHV68	A	I	D	A	*	S	F	I	K	N
HVS	A	I	D	A		G	F	I	K	D
EBV	A	I	D	A		G	F	L	R	N
EHV	A	I	D	A		G	F	I	K	N
AHV	A	I	D	A		G	F	I	S	N
HCMV	S	V	D	A		L	Y	I	R	K
SCMV	S	V	D	A		L	Y	I	Q	E
HHV-6	S	L	D	T		S	Y	I	R	K
VZV	A	V	N	N		M	L	L	R	D
HSV-1,2	A	V	N	N		M	M	L	R	D

Figure 1. Identification of an autolysis site within KSHV Pr. (a) Immunoblot of KSHV autolysis product during bacterial expression. Lane 1 contains wild-type KSHV Pr. Lane 2 contains the active site mutant S114A. Lane 3 contains the D-site mutant S204G. Relative molecular masses are indicated to the left of lane 1. Samples were harvested following five hours of protein expression after induction with 100 μg/ml IPTG. All samples contain 2 μl from a 200 μl boiling lysis in Laemmli buffer of the transformed X-90 *E. coli* pellet from 1 ml of identically treated and harvested cultures. Samples were run on a 12.5% polyacrylamide-SDS gel (Laemmli, 1970) and electrophoretically transferred to BA-85 nitrocellulose (Schleicher & Schuell). Anti-KSHV Pr serum (Ünal *et al.*, 1997) was incubated with the membrane at a dilution of 1:2000 and detected using enhanced chemiluminescence (Amersham). (b) Identification of KSHV Pr D-site by sequence analysis. Only residues spanning KSHV Pr positions 200-208 and hCMV residues 225-233 are depicted. Residues identical with the KSHV sequence are in red, indicating a high degree of sequence similarity with all of the proteases except those of the α-herpesviruses VZV and HSV-1 and 2 in this region. The cleavage site is indicated by an asterisk between KSHV Pr Ala203 and Ser204. EHV, equine herpesvirus; AHV, avian herpesvirus; SCMV, simian cytomegalovirus; HHV-6, human herpesvirus-6.

The size difference between KSHV Pr and its truncation product, Pr Δ, was 3 kDa as judged by SDS-PAGE/immunoblot analysis (Figure 1(a)). Sequence analysis identified a possible cleavage site 27 amino acid residues from the enzyme's C terminus; the loss of these 27 residues would convert the 25.2 kDa protein to a 22.2 kDa fragment. This region contained a preferred Ala-Ser bond at

positions 203 and 204, which aligns with the Ala228-Leu229 dipeptide of hCMV Pr (Figure 1(b)), but was not previously identified as an internal site (I-site) due to its location relative to those of hCMV. While hCMV Pr's two I-sites are present within loops (Chen *et al.*, 1996; Qiu *et al.*, 1996; Shieh *et al.*, 1996; Tong *et al.*, 1996) and do not significantly perturb proteolytic activity (Holwerda *et al.*, 1994; O'Boyle *et al.*, 1995), the I-site of KSHV Pr is predicted to be present within the conserved α-helix of the protease's dimer interface based on an homology model of the enzyme (Figure 2(a)). Due to this autolysis site's position and function within KSHV Pr, and to differentiate it from those of the hCMV enzyme, in this report it is referred to as the dimer disruption site (D-site).

Stabilization of KSHV protease to autolysis

In an attempt to reduce autolysis while maintaining an intact dimer interface, a conservative substitution was sought for the D-site of KSHV Pr. After examination of a multiple sequence alignment of this region of herpesviral proteases, a Ser → Gly (S204G) substitution at the P1′ position was suggested (Figure 1(b)). The only other protease of this class with an Ala-Ser dipeptide at this position is that of murine herpesvirus-68 (MHV68), which has not been characterized. The more closely related herpesvirus saimiri (HVS) enzyme differs, as does that of Epstein-Barr virus (EBV), at the P1′ position with Gly. While EBV Pr has activity in the range of other characterized enzymes of this class, it does not inactivate with an Ala-Gly bond at this site, even though the remaining residues are similar (Donaghy & Jupp, 1995). The degree of similarity between KSHV and hCMV Pr within this region is quite high, which is why hCMV Pr, rather than HSV or VZV Pr, was used as the basis for the model in Figure 2(a). HSV and VZV Pr differ significantly from the sequence of both KSHV and hCMV enzymes near the KSHV D-site, and demonstrate a different dimer interface contact pattern from hCMV Pr (Hoog *et al.*, 1997; Qiu *et al.*, 1997).

Construction of KSHV Pr S204G by PCR-mediated site-directed mutagenesis resulted in the reduction of autoproteolysis during bacterial expression (Figure 1(a)), and the purified variant retained full activity in a continuous fluorescence-based enzyme assay (Table 1). The migration of this enzyme by SDS-PAGE is identical with full-length wild-type KSHV Pr and the S114A variant, and little or no visible truncation product was present by immunoblotting in repeated expression trials. In addition, the k_{cat}/K_m value for wild-type KSHV Pr prior to autolysis is the same as that of the S204G variant, 1.1 (±0.4) × 10^3 M^{-1} min^{-1}, for the hCMV M-site substrate used previously (Holskin *et al.*, 1995; Ünal *et al.*, 1997). KSHV Pr S204G was also tested for reactivity against a newly synthesized fluorogenic KSHV R-site sub-

Table 1. Summary of kinetic data and apparent secondary structure

Protein	k_{cat}/K_M (10^3 M^{-1} min^{-1}) hCMV M-site[a]	KSHV R-site[b]	Apparent secondary structure content (%)[c] α-Helix	β-Strand	Other
KSHV Pr	1.1 (±0.4)	ND[d]	45	23	31
Pr S204G	1.1 (±0.4)	0.52 (±0.1)	45	23	31
Pr S114A	<0.01	ND[d]	45	23	31
KSHV Pr Δ	<0.01	ND[d]	24	24	53

The KSHV R-site was chemically synthesized: N-Fmoc-*L*-Glu-(EDANS)-OH was prepared similarly to methods previously described (Maggiora *et al.*, 1992). Synthesis was initiated with Boc-Gly-Merrifield resin, DABCYL was incorporated using orthogonally protected Boc-N$^{\alpha}$-Lys(N$^{\varepsilon}$Fmoc)-OH, and Fmoc was removed. DABCYL was activated in the presence of HOBT and coupled (Konig & Geiger, 1992), and the remainder of the peptide assembled using standard solid-phase methodology. N-Fmoc-*L*-Glu-(EDANS)-OH was coupled in the last step and Fmoc removed. The peptide was then cleaved and deprotected (Pennington, 1994). After purification by RP-HPLC in an aqueous TFA/MeCN gradient, the product was lyophilized and verified by amino acid analysis and ESI-MS. ESI-MS $(M + H)_{theory}$ 2107.48, $(M + H)_{obs}$ 2108.

Protein samples were purified after expression in *E. coli* strain X-90 grown in LB medium after five hours induction with 100 μg/ml IPTG at 30 °C. Following sonication and centrifugation in 50 mM potassium phosphate (pH 7.8), 25 mM NaCl, 1 mM DTT, 1 mM EDTA, streptomycin sulfate was added to a concentration of 1 %. $(NH_4)_2SO_4$ was added to 25 % saturation following centrifugation, the sample cleared, and protein precipitated by 45 % saturation with $(NH_4)_2SO_4$. After resuspension, protease was eluted from butyl sepharose FF resin (Pharmacia) using a 0.5 → 0 M $(NH_4)_2SO_4$ gradient in 50 mM potassium phosphate (pH 8), 25 mM NaCl, 1 mM DTT, 0.1 mM EDTA. Protease-containing fractions were dialyzed into 50 mM Tris (pH 8.0), 1 mM DTT, 0.1 mM EDTA, and eluted from a Mono-Q HR column (Pharmacia) in a 0 → 0.5 M NaCl gradient. Protease-containing fractions were dialyzed into assay buffer (25 mM potassium phosphate (pH 7.0), 25 % (v/v) glycerol, 150 mM KCl, 0.1 mM EDTA, 1 mM DTT or 2-mercaptoethanol), concentrated using YM-10 membranes (Amicon, Inc.), and fractionated over a Superdex-75 HiLoad 26/60 prep grade column (Pharmacia). Approximately 2-5 mg per liter culture of >99 % pure KSHV Pr were obtained for all variants as judged by Coomassie Brilliant Blue staining of SDS-PAGE gels. Protein concentrations were determined using a calculated $\varepsilon_{280} = 0.9$ ml mg^{-1} cm^{-1} for full-length molecules, and $\varepsilon_{280} = 1.0$ ml mg^{-1} cm^{-1} for KSHV Pr Δ.

Enzyme assays were performed at 20 °C in 0.5 ml assay buffer using a Fluorolog-3 fluorometer (ISA-SPEX). Product formation was monitored as described (Holskin *et al.*, 1995; Matayoshi *et al.*, 1990; Ünal *et al.*, 1997). The k_{cat}/K_m values were determined by a linear fit of [product]/time *versus* [substrate] in range of 1-25 μM with an enzyme concentration of 1 μM.

[a] hCMV M-site: (DABCYL)-Arg-Gly-Val-Val-Asn-Ala↓Ser-Ser-Arg-Leu-Ala-(EDANS).

[b] KSHV R-Site: Glu-(EDANS)-Val-Tyr-Leu-Lys-Ala↓Ser-Gln-Phe-Pro-Ala-Gly-Ile-Lys-(DABCYL)-Gly-OH.

[c] Secondary structure estimated using *k2d* software (Andrade *et al.*, 1993; Merelo *et al.*, 1994).

[d] ND, not determined.

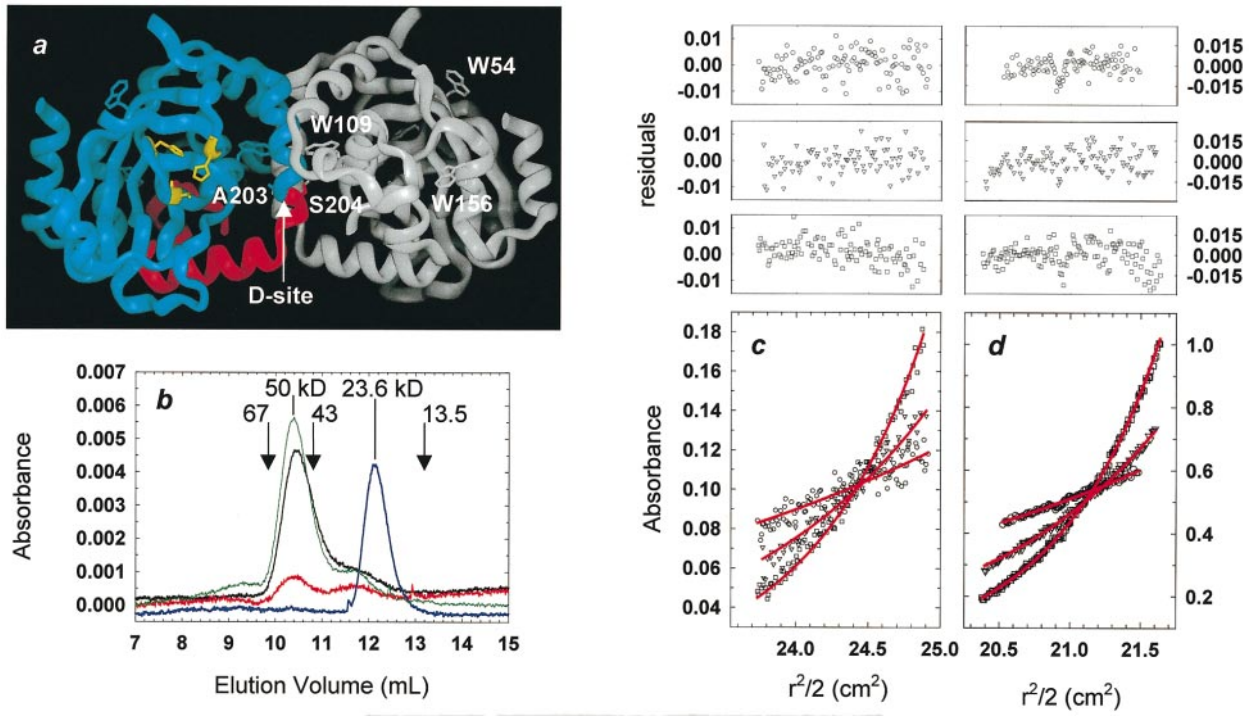


Figure 2. Effect of D-site cleavage on KSHV Pr dimerization. (a) Homology model of KSHV Pr dimer. Monomers are presented in gray and blue. The 27 amino acid fragment cleaved from one monomer upon D-site autolysis is depicted in red. Active site residues from this monomer are in yellow. Disordered loop regions from the hCMV structure were omitted from the model. The Ala203-Ser204 D-site junction is labeled, as are the three Trp residues of one monomer. The KSHV Pr homology model was based upon hCMV Pr (PDB accession code CMV1). Following a sequence alignment using pileup (GCG, Madison, WI) provided through the Computer Graphics Laboratory at UCSF, the residues of hCMV Pr differing from KSHV Pr were replaced using the Biopolymer module of the Insight software package (Molecular Simulations, Inc.). Insertions in hCMV Pr relative to KSHV Pr are present in disordered loop regions of the CMV1 structure: nine residues at the N terminus (within an 11 residue disordered region), 44-51 (within a 13 residue disordered region), 147-151 (within a 19 residue disordered region), and 203-204 (within an 11 residue disordered region). (b) Loss of dimerization upon D-site cleavage. Size-exclusion chromatography was performed with a Superdex-75 HR 1 cm × 30 cm column (Pharmacia). The 100 μl samples were loaded in assay buffer at a flow rate of 0.5 ml min^{-1} at room temperature. Full-length KSHV Pr at 5 μM (black) and S204G at 7.5 μM (green) exhibit dimerization. At 1.8 μM, KSHV Pr is roughly 1:1 dimer:monomer (red). The 12 μM KSHV Pr Δ exhibits no detectable dimerization, eluting at a monomeric molecular weight (blue). The column was calibrated with sizing standards, indicated by downward arrows. (c), (d) Sedimentation equilibrium analysis of KSHV Pr S204G dimerization. The absorbance data (230 nm) were collected at two total protein concentrations, 1 and 5 μM, respectively, and at three rotor speeds, 10,000 (circles); 15,000 (triangles); and 20,000 rpm (squares). The red curves represent simulated data derived from the best fit parameters of a monomer molecular mass of 20.5 kDa and a K_d of 1.7 (±0.9) μM. Residuals are plotted in separate panels above the Figures. The monomer mass was resolved as 20.5 kDa, lower than the prediction of 25 kDa, but similar to the value observed for hCMV Pr in analogous experiments (Cole, 1996). Attempts to fix the mass at the predicted value resulted in larger, significant deviations in the residuals of the fit (data not shown), and a 20-fold higher K_d. KSHV Pr S204G was dialyzed extensively against assay buffer, placed in a six chamber centerpiece with dialysis buffer serving as reference, and spun in a Beckman Optima XL-A. Equilibrium was verified after 20 hours at each rotor speed by overlaying four-hour-spaced radial absorbance scans. The data set was simultaneously analyzed by non-linear least-squares fitting using the Beckman-supplied package for Origin 4.1 software (Microcal Software, Inc.) using an ideal monomer-dimer assembly model. Sednterp 1.01 software (Hayes *et al.*, 1997) was used to estimate the protein's partial specific volume and the solvent density.

strate, and was found to hydrolyze this molecule at roughly half the rate, 0.52 (±0.1) × 10^3 M^{-1} min^{-1} (Table 1). This compound exhibited fivefold greater solubility and fluorescence enhancement than a previously utilized KSHV M-site substrate (Ünal *et al.*, 1997). Purified KSHV Pr Δ did not exhibit detectable peptidolytic turnover, consistent with the loss of proper active site geometry and/or substrate binding upon truncation; as expected, the active site variant S114A was also incapable of hydrolysis (Table 1).

Loss of dimerization upon KSHV protease D-site cleavage

Due to the D-site's presence within the predicted dimer interface of KSHV Pr (Figure 2(a)), the oligomerization properties of the enzyme were examined. At concentrations above 5 μM, purified full-length KSHV Pr was present predominantly as a dimer, eluting from a gel-filtration column with an estimated molecular weight of 50.1 kDa (Figure 2(b)). However, upon reduction of the total

protein concentration to 1.8 μM, a nearly 1:1 ratio of dimer to monomer was present, indicative of the sample being at a concentration near its K_d. On the other hand, isolated KSHV Pr Δ displayed no dimerization, either at concentrations in the 10 μM range as assayed by gel-filtration where it eluted with an M_r of 23.6 kDa (Figure 2(b)), or at nearly 0.1 mM levels as examined by analytical equilibrium ultracentrifugation (data not shown). From these experiments it became apparent that KSHV Pr D-site cleavage disrupted not only enzymatic activity but also dimerization.

The dimerization of the D-site S204G variant was analyzed using conditions identical with those for the wild-type enzyme. In the same gel-filtration assay KSHV Pr S204G dimers eluted at an M_r of 50.8 kDa, essentially identical with that of the non-truncated wild-type enzyme which eluted at 50.1 kDa (Figure 2(b)). Since KSHV Pr S204G was stabilized to autolysis, it was assayed by sedimentation equilibrium to further evaluate its dimerization properties. Simultaneous analysis of data collected at loading concentrations of 1 and 5 μM, and at three rotor speeds from 10,000 to 20,000 rpm, resulted in a K_d for S204G dimerization of 1.7 (±0.9) μM (Figure 2(c) and (d)). This value agreed with a previous analysis of hCMV Pr dimerization by similar means (Cole, 1996), and also coincided with the 1:1 dimer:monomer ratio of KSHV Pr at low micromolar concentrations (Figure 2(b)).

Conformational changes during autolytic inactivation of KSHV protease

In order to further understand the structural significance of proteolytic inactivation of KSHV Pr at its dimer interface, a spectroscopic characterization was performed. While the three intact, dimeric molecules exhibited nearly indistinguishable circular dichroism (CD) spectra, qualitatively similar to that of hCMV Pr (Liang *et al.*, 1998), KSHV Pr Δ suffered a greater than 60% loss in ellipticity (Figure 3(a)). A computational analysis of the spectral properties of KSHV Pr Δ was performed, yielding an apparent reduction in helical content relative to the full-length protease molecules (Table 1). While this finding is consistent with the cleavage site's presence within the dimer interface α-helix, firm conclusions regarding the exact nature of the secondary structural loss are difficult to resolve from such analyses. In particular, because of the novel α/β fold of the dimeric herpesvirus-type serine proteases, the established reference sets of protein CD spectra may not adequately represent the conformational changes taking place upon KSHV Pr D-site cleavage.

While the helical packing interactions contributing to KSHV Pr dimerization are lost upon D-site cleavage, it appears that the effects of truncation are not limited to the secondary structure within this protein-protein interface. An apparent loss of tertiary structure is evident in the 20% weaker, 10 nm red-shifted fluorescence emission spectrum of KSHV Pr Δ relative to full-length KSHV Pr (Figure 3(b)). Truncation of KSHV Pr at its D-site is not predicted to remove any Trp residues,

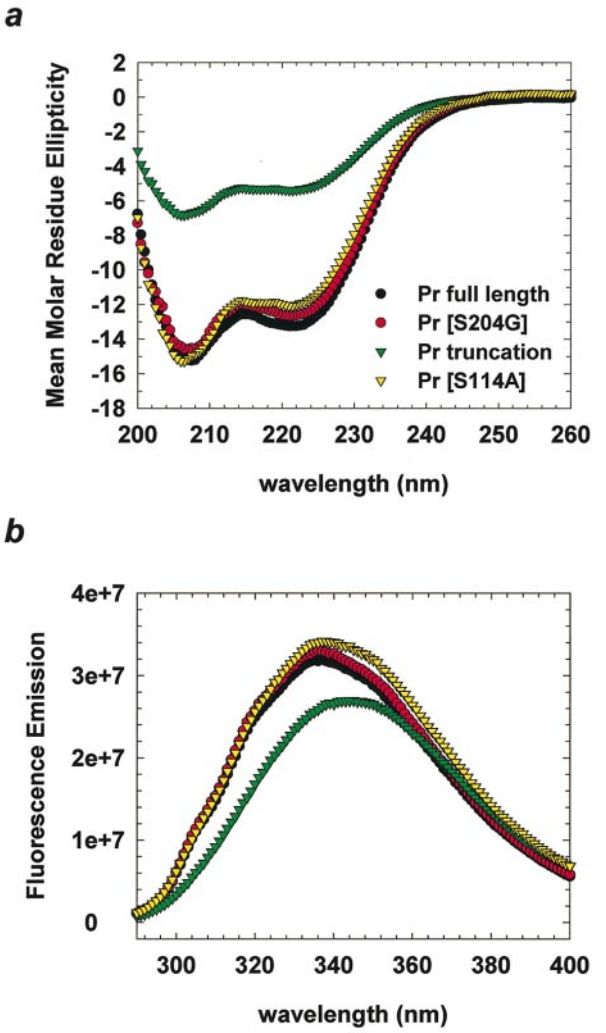


Figure 3. Spectroscopic detection of structural changes within KSHV Pr upon D-site cleavage. (a) Secondary structure loss detected by CD spectroscopy. All full-length protease molecules exhibit nearly identical CD spectra and secondary structures at 0.5 to 1.5 μM concentrations in assay buffer (Table 1). At the same concentration, KSHV Pr Δ has an altered spectrum, with a much reduced mean molar residue ellipticity (mdeg cm^2 $dmol^{-1}$ g^{-1}). CD scans were acquired on a Jasco 710 Spectropolarimeter; the samples held in 0.2 cm pathlength cuvettes at 20 °C, and were obtained by tenfold signal averaging and solvent correction. (b) Tertiary structural changes or more highly solvent-exposed hydrophobic core of KSHV Pr Δ detected by intrinsic protein fluorescence. Protein samples were at 1 μM concentration in assay buffer at 20 °C. All full-length protease molecules have coincident spectra, while KSHV Pr Δ differs significantly in magnitude and the wavelength of peak emission intensity. Fluorescence excitation was at 275 nm, and emission (cps/μAmp) monitored by five-fold averaged, solvent and lamp intensity-corrected scans.

although the P2′ Phe of the D-site is lost, as well as the Tyr residue four positions from the C terminus of the enzyme. Neither of these amino acid residues likely contribute much to the overall fluorescence emission of KSHV Pr. Their low experimental fluorescence sensitivities relative to Trp preclude this (Tyr, 2.0×10^{-2}; Phe, 0.08×10^{-2}; Trp, 11.0×10^{-2} M^{-1} cm^{-1}; Cantor & Schimmel, 1980), especially since all three Trp residues and the remaining seven Phe and four Tyr side-chains are retained in KSHV Pr Δ. Rather, in the homology model of the enzyme, it appears that two of the three Trp residues within each KSHV Pr monomer become more solvent-exposed upon truncation. Trp109, corresponding to hCMV Pr Ser127, is directly behind the dimer interface α-helix of its own monomer, and KSHV Pr Trp156, conserved in hCMV Pr at position 179, contacts residues near the C terminus of the protease which are lost upon autolysis (Figure 2(a)). On the other hand, KSHV Pr Trp54, which aligns with hCMV Pr His71, is near neither the dimer interface nor residues lost upon truncation. This may indicate that the red-shift of the entire emission spectrum is due to partial relaxation of the hydrophobic core of the protein. However, the likely maintenance of apparent secondary structure within KSHV Pr Δ (Figure 3(a), Table 1), coupled with its cooperative unfolding at relatively high temperature (A.M.N., V. Dötsch & C.S.C., unpublished observations), seems to preclude the wholesale loss of tertiary packing of the remaining α-helical and β-sheet elements.

Possible role of D-site cleavage in regulation of KSHV maturation

The inactivation of KSHV Pr by disruption of its dimer interface, and the resultant structural transitions which give rise to the altered spectroscopic signature of the enzyme, provide an interesting and novel mechanism for the regulation of viral protease activity (Babe & Craik, 1997). By selectively perturbing essential protein-protein contacts distal from its active site, KSHV Pr transmits structural information to its catalytic residues and/or substrate binding determinants (Bonneau *et al.*, 1997; Tong *et al.*, 1998). The precise role of the inactivation event in the viral life cycle is yet to be determined, although recent evidence suggests the presence of KSHV Pr Δ in maturing viral particles produced in culture (T.R.P., M. Lagunoff, D. Ganem & C.S.C., unpublished observations).

Perhaps KSHV Pr activity is subject to different regulatory mechanisms than related enzymes due to unknown requirements of KSHV maturation. The only other herpesviral proteases with known I-sites are those of the cytomegalovirus lineage. hCMV Pr contains two I-sites whose cleavage has no apparent biochemical consequence in terms of regulating enzyme activity, although autolysis at one of these sites does perturb crystallization of the enzyme (Chen *et al.*, 1996; Holwerda *et al.*, 1994; O'Boyle *et al.*, 1995; Qiu *et al.*, 1996; Shieh *et al.*, 1996; Tong *et al.*, 1996). While KSHV Pr is most closely related to HVS Pr, the latter enzyme does not contain the Ser preferred for cleavage at P1′ of the KSHV D-site (Figure 1(b)). The fact that MHV68 Pr does contain this site, even though this virus is more distantly related to KSHV, may indicate selective pressure governing such a cleavage event. The role of D-site cleavage will be further addressed in subsequent cell culture and high-resolution structural studies of KSHV Pr and Pr Δ, as will the linkage between dimerization and activity. The functional coupling between KSHV Pr's dimer interface and catalytic machinery, as probed in this study using the autolysis site as an experimental tool, provides strong evidence for the positive regulatory role of oligomerization in this class of enzymes.

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