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HIV-1 Protease Cleaves Actin During Acute Infection of Human T-Lymphocytes

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ABSTRACT

Actin, one of the most abundant proteins of the cell, is hydrolyzed by the human immunodeficiency virus type 1 (HIV-1) protease during acute infection of cultured human T lymphocytes. The actin fragments produced during the course of infection are identical to those obtained by recombinant HIV-1 protease digests of (1) a lysate from uninfected T lymphocytes and (2) globular actin itself. Hydrolysis by the HIV-1 protease of physiologically important host cellular proteins during infection may have important consequences relative to viral pathogenesis.

INTRODUCTION

Retroviral proteases are generally believed to function only within the confines of the budded viral particle, where they are essential for processing the *gag* and *gag/pol* polyproteins into the structural proteins and enzymes of the mature, infectious virus.[1] This makes sense because premature processing of the viral fusion proteins within the host cell would abort the viral life cycle and halt infectivity.[2] Moreover, because the retroviral proteases are obligate dimers,[3–6] they appear to be endowed uniquely for regulation by concentration-dependent activation. The transition from the host cell to the budded immature viral particle is attended by an enormous concentration of the *gag* and *gag/pol* polyproteins,[7] and this would greatly favor the chance encounter of two 160 kD *gag/pol* precursors, each housing a single protease unit, to produce an active dimer within this macromolecular assembly capable of initiating a processing cascade.

Despite the teleological and physicochemical arguments for restricting the activity of retroviral proteases to viral particles, recent evidence suggests that the protease from human immunodeficiency virus type 1 (HIV-1 protease) can exhibit activity within the host cell. Kaplan and Swanstrom[8] have demonstrated that processing of the *gag* proteins occurs, at least in part, in the host cell cytoplasm. In addition, Rivière et al.[9] have shown that HIV-1 protease is able to cleave the precursor of NF-κB during acute infection of T lymphocytes.

We have documented a number of nonviral proteins that can serve as substrates of the HIV proteases. Some of these, including calmodulin,[10] troponin C,[11] prointerleukin 1β,[11] Alzheimer amyloid precursor protein,[11] and actin[11] play important structural and regulatory roles in cellular metabolism, and we have speculated as to the consequences of activation of the HIV protease within the infected cell relative to destruction of these proteins.[10,11] Here we show that actin is hydrolyzed by the HIV-1 protease in acutely infected T lymphocytes.

RESULTS

In Figure 1A is shown a two-dimensional gel electrophoretic separation of fragments produced by HIV-1 protease cleavage of actin. Spots marked with arrows a–g were demonstrated to be actin derived; others seen in Figure 1A are contaminants of the actin sample and are seen in a control of undigested actin. The 2-D gel displayed in Figure 1B is that of a control sonicate of human T lymphocytes (cell line A3.01) that was incubated at pH 7.0 for 35 min at 30°C. Although actin clearly is present in this control, even after 22 h of incubation there was no appearance of any spots corresponding to the expected actin fragments defined

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in Figure 1A. However, incubation of the same sonicate from human T lymphocytes with recombinant HIV-1 protease[12] results in a substantial disappearance of actin within 2 h. More important, as shown in Figure 1C, addition of the HIV protease results in the appearance of spots which correspond to those generated during hydrolysis of actin (Fig. 1A). These results indicate that the fragments generated from a T-lymphocyte sonicate correspond to those produced from actin itself, and are not present in gels of the control.

The next question was to determine whether actin breakdown might be demonstrable in HIV-infected lymphocytes. The series of 2-D gels shown in Figure 2 A–D establish that this is the case. Figure 2A presents a control 2-D map of a lysate from freshly prepared A3.01 lymphocytes (noninfected) and 2B is the same sample spiked with the actin digest described in Figure 1A. This comparison establishes the migration of the actin fragments produced by digestion with the HIV-1 protease relative to that of the cell proteins. Figure 2C is a 2-D map of a lysate from HIV-1 (LAV-1_{BRU} strain) infected cells, prepared 9 days postinfection. Four of the new protein spots seen in this map correspond to four of the major actin fragments depicted in Figures 1A and 2B. The putative actin spots of Figure 2C can be darkened by adding to this infected cell lysate the HIV-1 protease digest of pure actin (Fig. 2D).

Considered together, the results of Figures 1 and 2 provide compelling evidence that host cell actin is broken down during the course of infection of T lymphocytes by HIV-1. It is important to note that we are not looking at a single product, but a series of peptides with a well-characterized pattern. Faint spots corresponding to actin peptides e and f appear in control cells (Fig. 2A), but they do not increase with time (i.e., they appear to be background proteins. No such background was seen for fragments a and d, again strengthening the rationale of looking at a series of products rather than any single peptide. This production of actin fragments in HIV-infected cells is not evident in noninfected cells. Control sonicates incubated for a period of up to 22 h do not show *any* of the series of fragments produced by cleavage of actin by the HIV protease. This cleavage pattern is distinct. We have described the course of actin hydrolysis by the HIV-1 and 2 proteases,[11] and the sites of cleavage are in a region of the molecule separate from that which undergoes digestion with the usual set of proteinases such as one would expect to find among the array of host cell enzymes.[13] Thus, it would seem most likely that the breakdown of actin during HIV infection is directly linked to the action of the viral protease. Clearly, there are consistent changes in other protein spots that are linked with HIV infection; some disappear and some are generated. We are continuing attempts to identify the nature of these changes.

Actin is, of course, one of the abundant proteins of the cell, being present in nearmillimolar concentrations. It plays an essential role in muscle and cell motility, and actin fibrils help to maintain the shape of the cell. The location of these elements near the cell surface would suggest a proximal disposition relative to the surface-oriented viral polyproteins as they aggregate prior to budding from the host cell. Thus, if low levels of HIV protease activity can arise from this macromolecular segregation within the cell, actin would be a nearby target and its hydrolysis could augment the process of exocytosis. Another view might be that the protease injected into the host following viral infection could be active and have access to membrane-associated proteins. The susceptibility of other structural proteins such as vimentin and desmin[14] and microtubule-associated proteins[15] to breakdown in vitro by the HIV protease have also been reported, although these processes have not been correlated with viral infection.

DISCUSSION

The findings reported herein, and those of Kaplan and Swanstrom[8] and Rivière et al.[9] pose intriguing questions with regard to the possible role of the HIV protease in the progression

FIG. 1. Two-dimensional gel analysis on large format 2-D gels (ISO-DALT® System, Hoefer Scientific) of: (A), fragments produced by cleavage of rabbit muscle actin (Sigma; identical to human actin) with the HIV-1 protease. Native actin from rabbit muscle at a concentration of 0.4 to 1.2 mg/ml (10 to 30 μM) in 4 mM MOPS buffer, pH 7.0, was incubated at room temperature with 0.2 to 0.6 μg of HIV-1 protease (total volume 250–500 μl). Reactions were carried out in the presence of a 2- to 12-fold molar excess of Ca^{2+}/ATP (with respect to actin), conditions that favor the globular, monomeric form of actin, called G-actin. A sample was withdrawn at 6 hours and the proteins were separated on 2-D gels[25] which were stained with a polychromatic silver stain.[26] Spots designated by arrows a–g were shown to be derived by protease digestion of actin (open arrow); other spots are contaminant proteins seen as background. (B) A control sonicate of human T-lymphocyte cell line A3.01[27] following incubation at 30°C in 0.1 M MOPS buffer, pH 7.0, for 35 min. Cell line A3.01 is a CEM-derived, leukemic T-cell line expressing CD4; growth was in culture medium RPMI from Quality Biological, supplemented with L-glutamine, HEPES buffer, 10% fetal bovine serum, and Pen-Strep antibiotics. Cells (35 mg wet cell wt) were sonicated in 60 μl of buffer and incubated at 30°C with 25 μg of HIV-1 protease. After 35 min, 2, 7, and 22 h samples were withdrawn and analyzed by 2-D gel electrophoresis, only 35-min samples are shown here. (C) Same as in (B), but in the presence of recombinant HIV-1 protease. Spots a–f are not present in the control sonicate (B), but correspond to those derived from actin (A).

FIG. 2. Two-dimensional gel analysis (see legend to Fig. 1): (A) Control A3.01 whole cell lysate with open arrow marking actin. Freshly harvested cells were washed in fresh Culture medium and lysed in solubilization buffer containing 9 M urea.[25] Cells, 100 mg wet wt, were diluted with 1 ml of lysis buffer, and 15 μl was loaded on to the gel. (B) Same as (A), but spiked with about 5 μg of actin fragments prepared as described in Fig. 1A. Arrows indicate positions of actin fragments a–f, not seen in the control (A). (C) A3.01 cells harvested and lysed as in (A), but after 9 days infection with LAV-1_{BRU} strain of HIV-1 propagated in human PBL. Virus was added at an MOI of 0.003, and cells/inoculum were incubated at 37°C, 5% CO_2 for 1 h with agitation every 15 min. Cells were resuspended in culture medium at 1×10^6/ml and observed daily for cytopathic effects (cpe). Appearance of significant cpe was generally noted 7–9 days postinfection.[28] Spots a, d, e, and f corresponding to actin fragments are visible following HIV infection. These are highlighted as shown in panel (D) by addition of the same mixture of fragments added to (B).

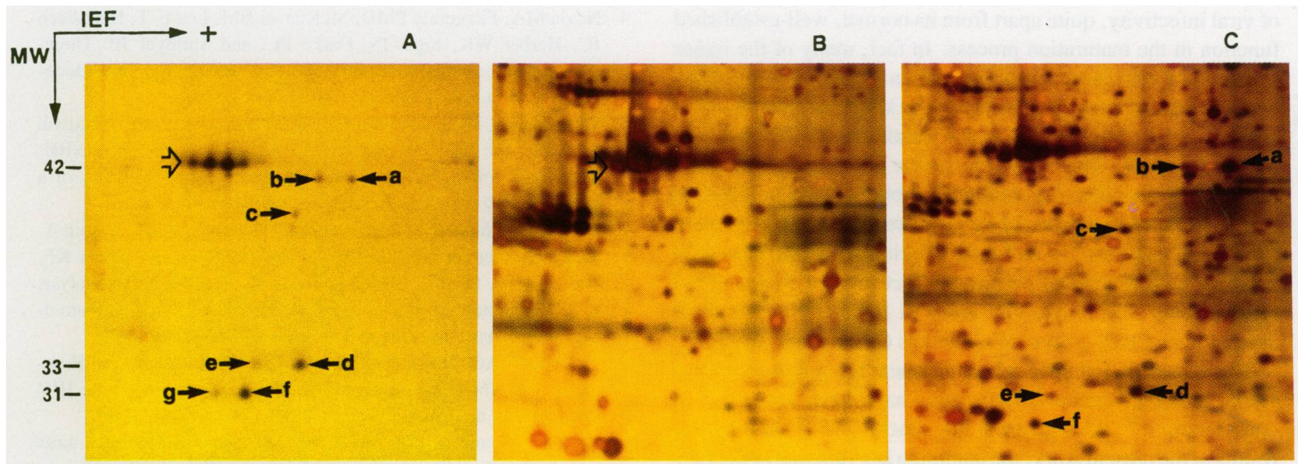

FIG. 1

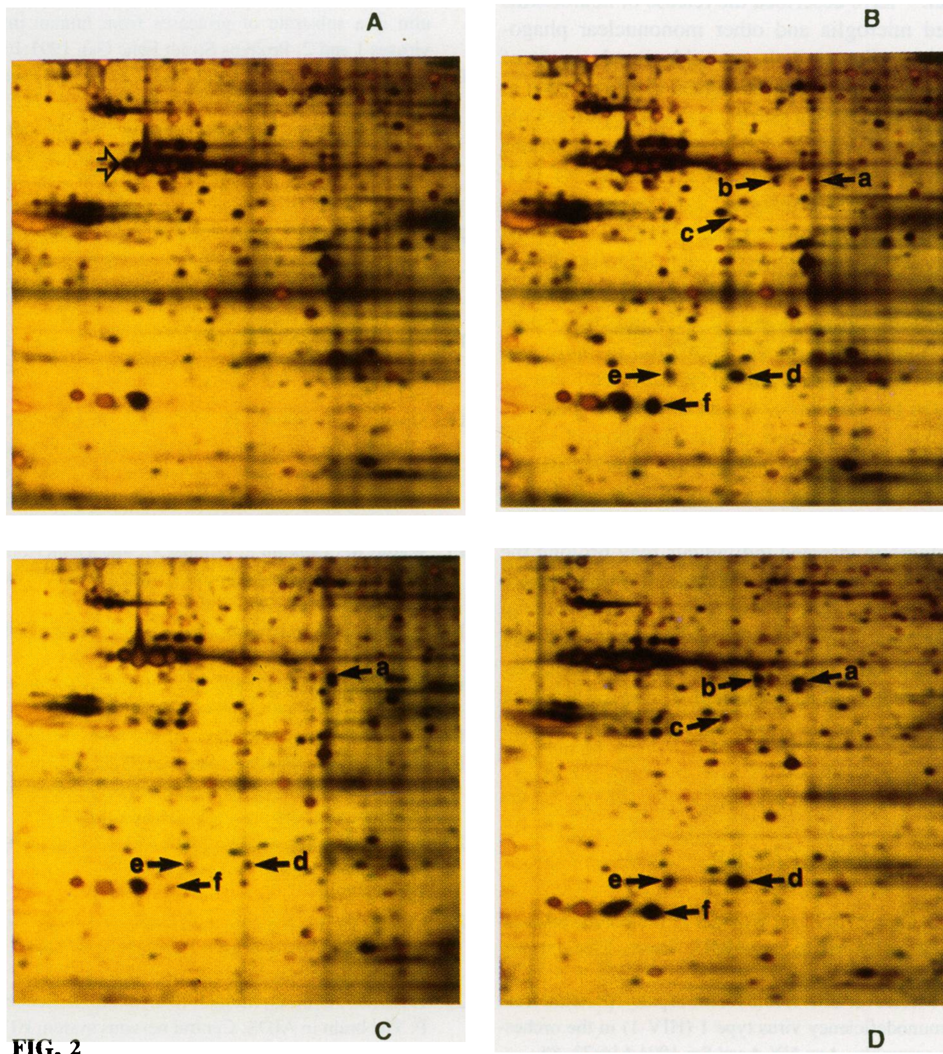

FIG. 2

of viral infectivity, quite apart from its normal, well-established function in the maturation process. In fact, many of the issues surrounding retroviral infections remain shrouded in mystery, and it is now of even greater interest to know whether the viral protease might be involved in the breakdown of needed host proteins, or in the liberation of bioactive and possibly immunomodulatory peptides. For example, subtle defects in T-helper cell function can be detected long before a critical reduction in $CD4^+$ cell numbers (16 and refs. therein). This impairment in T-helper cells is evident despite the fact that the frequency of HIV-infected cells ranges between 1 in 100 and 1 in 10,000 in asymptomatic HIV-infected individuals (17 and refs. therein).

The causes of T-helper cell impairment are not at all understood, although various mechanisms have been proposed for consideration.[16] As suggested by others[8,9,18,19] and by the results of the present work, the inappropriate function of the HIV protease may provide yet another mechanism to account for this early compromise in T-helper cell viability. Indeed, our demonstration that calmodulin[10] and troponin C[11] are excellent substrates of the viral protease may help explain defects seen in calcium metabolism accompanying HIV infection.[20] Interestingly, Giulian et al.[21] have described the release of neurotoxins from HIV-infected microglia and other mononuclear phagocytes. These workers discussed the possible involvement of these neurotoxins in causing the neurological disorders seen in many AIDS patients. It is known that these disorders are not the result of immunosuppression.[22] Although these neurotoxins have yet to be identified as peptides, or as products of HIV protease action, the aberrant proteolytic excision of bioactive fragments by the viral enzyme offers a plausible hypothesis that can be tested easily. Finally, we have shown that prointerleukin 1β undergoes cleavage in the prosegment by the HIV protease.[11] The possible processing of cytokines in HIV infection could have widespread ramifications in the regulation of numerous biological pathways and in T-cell growth and differentiation.

In conclusion, the possible involvement of the HIV protease in destruction of host cell function and in liberation of signal peptides that can influence viral expression and/or act as immunosuppressants adds to the importance of this target in the treatment of AIDS. Moreover, blocking the activity of this enzyme in asymptomatic infected individuals may prolong the latency period in the progression of the disease. It is important to note that although the inhibition of the viral protease results in the arrest of HIV infectivity in cell cultures[23,24] the protease has not yet been proven to be a viable target in treatment of AIDS. The coming months will witness clinical testing of protease inhibitors, and we hope that they may show beneficial properties.

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