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The catalytic properties of the recombinant reverse transcriptase of bovine immunodeficiency virus

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Abstract

Bovine immunodeficiency virus (BIV) is a lentivirus with no proven pathogenesis in infected cattle. Yet, in experimentally infected rabbits, it causes an AIDS-like disease. Consequently, we expressed two recombinant isoforms of BIV reverse transcriptase (RT), which differ in their C-termini, and studied their catalytic properties. Both isoforms prefer Mg^{+2} over Mn^{+2} with most DNA polymerase and ribonuclease-H substrates. The processivity of DNA synthesis by the BIV RTs is higher than that of HIV-1 RT, whereas the fidelity of synthesis is even lower than that of the HIV-1 enzyme. The ribonuclease-H cleavage pattern suggests that the spatial distance between the polymerase and ribonuclease-H active sites of the two BIV RT isoforms equals 20 nt, unlike the 17 nt distance observed in almost all other RTs. The longer BIV RT version is somewhat less active than the shorter version, suggesting that the extra 74 residues (with homology to dUTPases) might obstruct efficient catalysis.

Keywords: Bovine immunodeficiency virus; Retrovirus; Reverse transcriptase; Lentiviruses; Human immunodeficiency virus; DNA polymerase; Ribonuclease H; Protease

Introduction

The bovine immunodeficiency virus (BIV) is a member of the lentivirus subfamily of retroviruses, a group of exogenous, nononcogenic viruses that cause chronic, multi-system disease in susceptible hosts (Gonda et al., 1987; Narayan and Clements, 1989). BIV was first isolated from a dairy cow with persistent lymphocytosis, lymphoid hyperplasia, central nervous system lesions and progressive weakness (Van der Maaten et al., 1972). Serological studies indicate that BIV is present worldwide (Cockerell et al., 1992; Forman et al., 1992), seropositivity for BIV has been correlated with decreased milk production in dairy cattle (McNab et al., 1994). An increased incidence of encephalitis and secondary bacterial infections has been reported in herds with high BIV seroprevalence (Snider et al., 1996). However, there is no conclusive evidence that BIV is the direct cause for the etiology of these syndromes. Such a demonstration is complicated by the co-infection with bovine leukemia virus (BLV), another retrovirus belonging to the HTLV/BLV group (Flaming et al., 1993, 1997). Lentivirus infections are typically characterized by the establishment of a persistent, lifelong infection and a slow, progressive disease course in the naturally infected host. There is wide variation in clinical disease both among and within the different members of the lentivirus subfamily. The pathogenesis of BIV still remains unknown in infected cattle and buffaloes, as no virus-specific diseases have been identified in acutely infected herds. Interestingly, BIV induces in experimentally infected rabbits clinical syndromes, including fatal immune dysfunctions similar to AIDS induced in humans by human immunodeficiency virus (HIV), in monkeys or in cats by the simian or feline immunodeficiency viruses, respectively (SIV and FIV) (Kalvatchev et al., 1995, 1998).

BIV resembles other lentiviruses in certain aspects of its pathogenesis, ultrastructure, genome organization and infectious cycle in susceptible cells (Braun et al., 1988; Garvey et al., 1990; Gonda et al., 1987). This nonprimate lentivirus resembles

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human and nonhuman primate immunodeficiency viruses and was, therefore, named BIV to reflect its genetic relationship and biological similarity to HIV (Gonda et al., 1987). Nevertheless, BIV is unable to infect human cells (Gonda et al., 1994). In terms of genome organization, BIV is the most complex nonprimate lentivirus. In addition to the obligate retroviral *gag*, *pol* and *env* structural genes, BIV encodes six putative nonstructural/accessory genes; *vif*, *tat*, *rev*, *vpw*, *vpy* and *tmx* (Garvey et al., 1990; Gonda, 1994, 1987; Oberste and Gonda, 1992; Oberste et al., 1991, 1993). Genetic variability is an outstanding feature of lentiviruses, contributing to the pathogenesis of these viruses by enabling them to persist in the host and evade anti-retroviral treatments. As shown for known lentiviruses, isolates of BIV exhibit a striking genomic diversity, located mainly in the viral envelope gene (Meas et al., 2001). However, assessment of the extent of variation in the reverse transcriptase (RT) domain of the *pol* gene revealed that this is one of the most conserved regions of the genome. The RT region of BIV genome, like that of HIV, is subjected to purifying selection

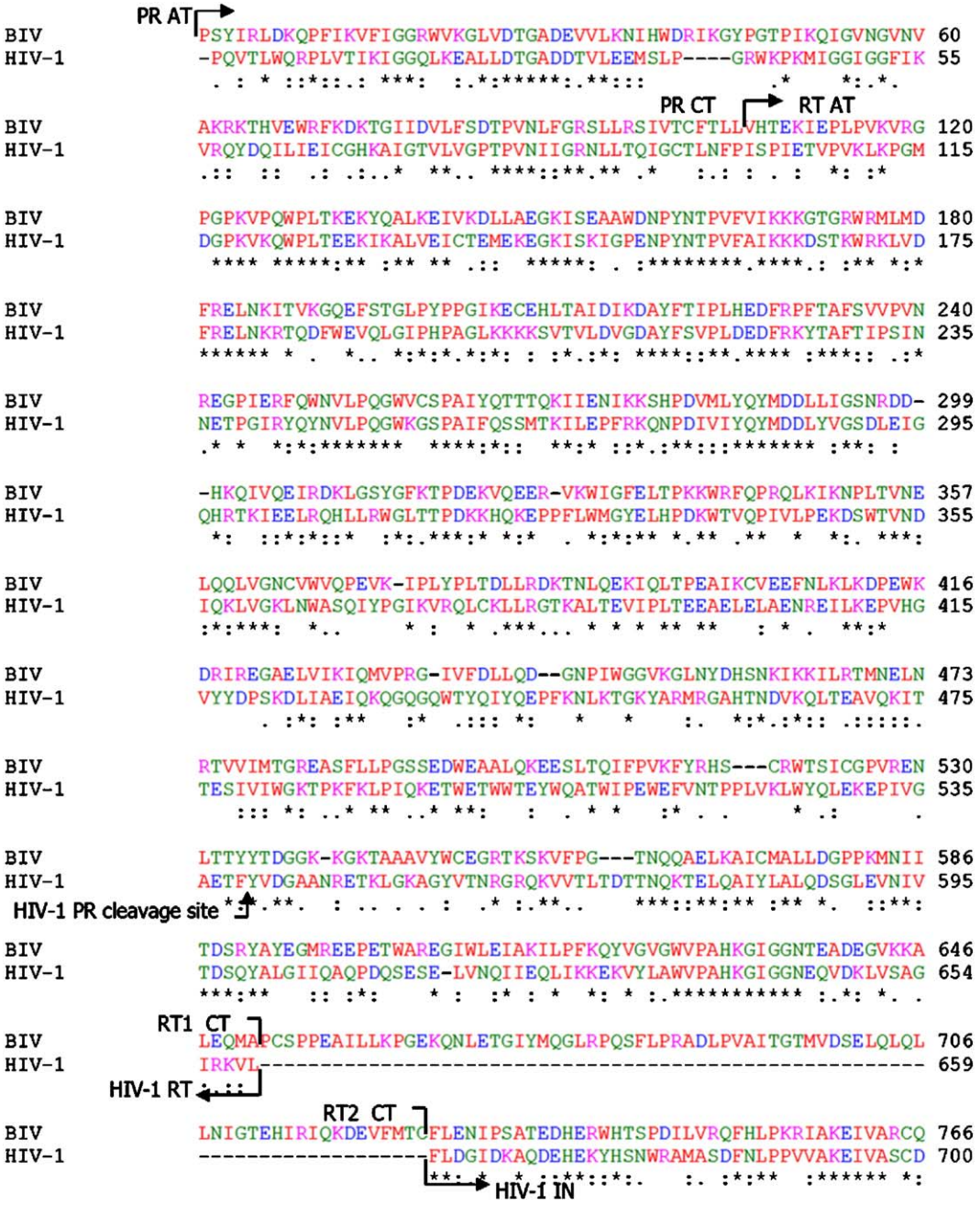

Fig. 1. Clustal W pairwise alignment of the amino acids residues encoded by the *pol* genes of BIV and HIV-1 (of the BIV127 and BH-10 strains, respectively). The upper lines represent the amino acids sequences of BIV PR and RT (and the amino-terminus of integrase). Recombinant BIV PR is 105 residues-long and recombinant RTs are 546 and 620 residues-long. BIV RT1 carboxyl terminus aligns with HIV-1 RT carboxyl terminus, RT2 carboxyl terminus is the last residue in the BIV sequence before the alignment with the amino terminus of HIV-1 IN. Asterisks in the lower lines mark the identical amino acids. The numbers of the residues are indicated on the left of each line. (AT) denotes amino terminus and (CT) denotes carboxyl terminus.