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Vpu-mediated CD4 down-regulation and degradation is conserved among highly divergent SIV_{cpz} strains

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Abstract

Human immunodeficiency virus type 1 (HIV-1) along with simian immunodeficiency viruses from chimpanzees (SIV_{cpz}) and three species of Old World monkeys from the genus Cercopithecus have been shown to encode a Vpu protein. To date, the functional characterization of Vpu has been limited to a small number of subtype B and more recently subtype C Vpu proteins. Using a recently developed VpuEGFP reporter system, we have shown that the subtype B and C Vpus are capable of preventing CD4 from being expressed on the cell surface. Using the same reporter system, we report here on the expression and functional analysis of Vpu protein from four SIV_{cpz} isolates (CAM13, ANT, TAN1, and GAB1). All four SIV Vpu fusion proteins were efficiently expressed and prevented CD4 expression on the cell surface and induced CD4 degradation. This was surprising as three of the SIV_{cpz} Vpu fusion proteins had only one canonical casein kinase II (CK-II) site (CAM13, ANT, TAN1) while previous studies with laboratory adapted HXB2 had indicated that both CK-II sites were required for CD4 degradation. Both ANT and TAN1 Vpu sequences encoded five consecutive negatively charged amino acids residues following the only CKII site (**SAIE**EDEE for ANT; **SGVE**EDEE for TAN1). We thus explored the possibility that this stretch of negatively charged amino acids might substitute for the lack of second CK-II site. Substitution of the aspartic acid at position 61 and glutamic acid at position 63 in the SIV_{cpz} ANT Vpu within with lysine residues abolished the ability of this protein to down-modulate cell surface expression of CD4. Similarly, change of a serine to an alanine residue following the single consensus CK-II site of the CAM13 Vpu (**SGNE**SDGGEEE) abolished CD4-down-regulation, suggesting that this serine was phosphorylated in the absence of a canonical CK-II site. Our results indicate that the serine was required, suggesting that this serine was phosphorylated by CK-II or possibly another cellular kinase. Taken together, these results show for the first time that Vpu proteins from SIV_{cpz} isolates, although quite diverse in sequence and predicted secondary structure from the HIV-1 subtype B protein, are capable of down-regulating CD4, which is one of the major functions of the HIV-1 protein.

Keywords: Vpu; SIV_{cpz}; CD4 down-regulation; HIV-1

Introduction

Human immunodeficiency virus type 1 (HIV-1) encodes for a small membrane bound protein Vpu, which has been shown to be encoded from the same mRNA that encodes the envelope glycoprotein precursor (Hout et al., 2004; Schwartz et al., 1990). Studies on the Vpu protein from HIV-1 subtype B isolates have shown that Vpu is localized predominantly to the Golgi complex and has two important functions within the virus infected cell (Kimura et al., 1994; Pacyniak et al., 2005; Strebel et al., 1989). One is the enhancement of virion release from infected cells, which has been associated with the transmembrane domain (Bour et al., 1995; Klimkait et al., 1990; Schubert et al., 1996a, 1996b). The second function of

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the Vpu protein is the down-modulation of CD4, which is believed to reside within the cytoplasmic domain of the protein (Willey et al., 1992; Vincent et al., 1993). Both of the predicted alpha-helical domains, the transmembrane domain, and the phosphorylation of the serine residues within the casein kinase II sites of the cytoplasmic domain have been shown to be essential to the CD4 down-regulation property of Vpu (Paul and Jabbar, 1997; Tiganos et al., 1998). In addition to cell culture studies, the Vpu protein is also a determinant of in vivo primate lentiviral pathogenicity. Using the simian-human immunodeficiency virus (SHIV)/macaque model, we have shown that an intact Vpu contributes to the rapid loss of circulating $CD4^+$ T cells in macaques infected with these pathogenic SHIVs (McCormick-Davis et al., 1998; Singh et al., 2001, 2003; Stephens et al., 2002).

Although the majority of the simian immunodeficiency viruses (SIV) lack a *vpu* gene, this gene is also encoded by SIVs naturally infecting chimpanzees (SIV_{cpz}), greater spot-nosed monkeys (SIV_{gsn}), mona monkeys (SIV_{mon}), and mustached monkeys (SIV_{mus}) (Barlow et al., 2003; Courgnaud et al., 2002, 2003; Santiago et al., 2002). However, the predicted amino acid sequence of the Vpu proteins from these various SIVs, including SIV_{cpz} strains from central (*Pan troglodytes troglodytes*) and eastern (*Pan troglodytes schweinfurthii*) chimpanzees, while retaining one or more consensus casein kinase II motifs in the cytoplasmic domain, are highly divergent from each other as well as from HIV-1 group M Vpu sequences. Thus, it remains unknown whether these structural homologues of the HIV-1 Vpu have the same functions (CD4 down-modulation and enhancement of virion release).

Recently, we developed a reporter system that permits analysis of intracellular transport and CD4 down-regulation by Vpu (Pacyniak et al., 2005; Singh et al., 2003). In this study, we have used this reporter system to analyze the intracellular transport and CD4 down-regulating function of four SIV_{cpz} Vpu proteins. We show that despite their sequence divergence from each other as well as HIV-1 group M, N, and O isolates, these four SIV_{cpz} Vpu proteins were capable of down-modulating cell surface expression of human CD4.

Results

Predicted secondary structure and phylogenetic analysis of HIV-1 and the SIV_{cpz} Vpu proteins

The secondary structure of the SIV_{cpz} Vpu proteins were compared to the well-studied subtype B Vpu protein. As shown in Fig. 1, the subtype B and C Vpu proteins as well as all four of the SIV_{cpz} Vpu proteins had a predicted transmembrane domain at the N-terminus of the protein. As previously reported, the cytoplasmic domain subtype B Vpu protein had a predicted secondary structure which consisted of two α-helical regions that were separated by a random coil region (Schubert and Strebel, 1994; Schubert et al.,

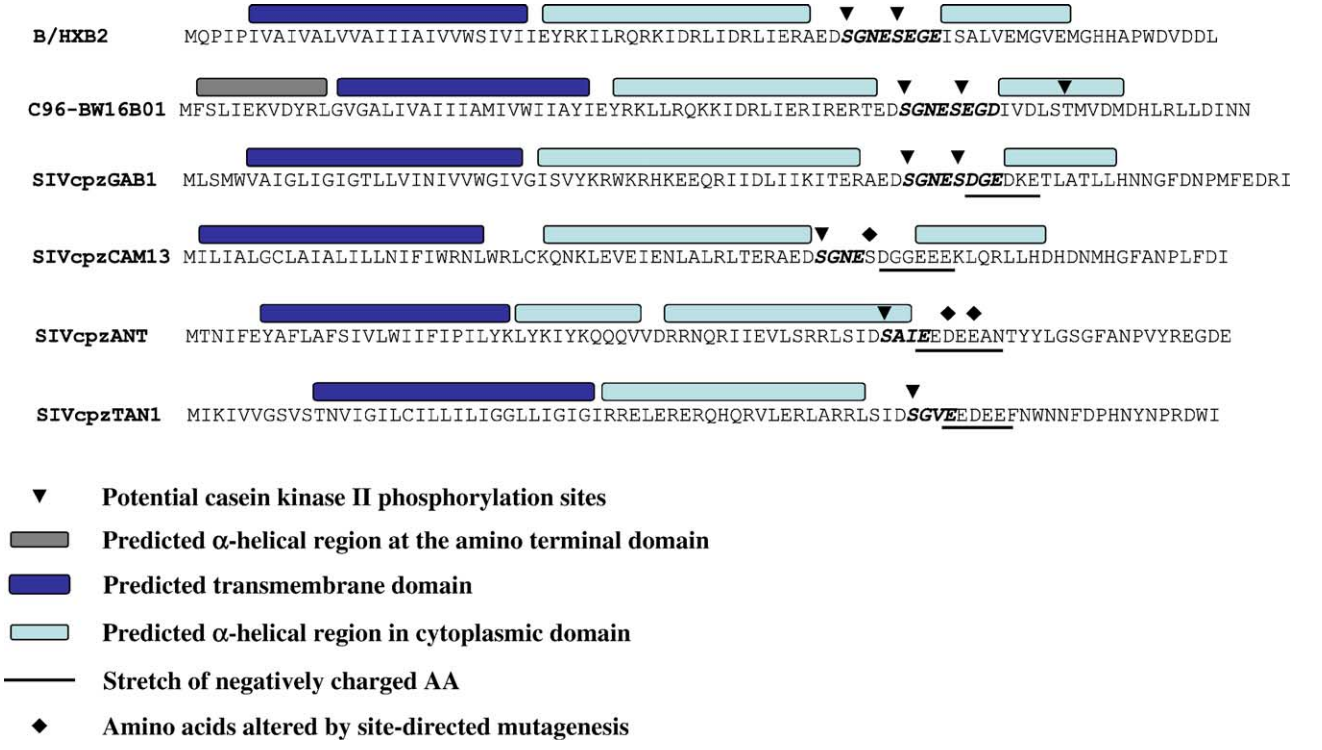

Fig. 1. The sequence and predicted secondary structure of the Vpu proteins analyzed in this study. Sequence of the Vpu proteins analyzed with the isolate name to the left of the sequences. Those residues bolded and in italics represent the potential casein kinase II sites. The residues of the SIV_{cpz} ANT and SIV_{cpz}CAM13 sequences with a diamond above them correspond to residues that were altered by site-directed mutagenesis as described in the text.