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Short communication

Glutamic Acid Rich Helix II Domain of the HIV-1 Vpu has Transactivation Potential in Yeast

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The transactivation potential of HIV-1 Vpu was identified from the yeast two-hybrid screening process. The helix II domain of HIV-1 Vpu protein and mutant Vpu protein lacking the transmembrane domain exhibited transactivation of the *LacZ* and *Leu2* reporter genes carrying LexA upstream activating sequences, but full-length HIV-1 Vpu and the helix I domain of HIV-1 Vpu did not. The helix II domain of HIV-1 Vpu consists of a number of acidic amino acids, and is especially rich in glutamic acid, a characteristic of many transcription factors. This result suggests that protein-protein interaction may occur through the acidic helix II domain of HIV-1 Vpu.

Keywords: Acidic domain, Helix II domain, HIV-1, Transactivation, Vpu.

Introduction

Vpu is a 16 kDa integral membrane phosphoprotein encoded by human immunodeficiency virus type I (HIV-1) that can form oligomeric structures *in vitro* and *in vivo* (Strebel *et al.*, 1988; 1989; Maldarelli *et al.*, 1993). It is associated predominently with the Golgi complex (Klimkait *et al.*, 1990), but is also present in association with the plasma membrane (Friborg *et al.*, 1995). The protein is 80 to 82 amino acids long depending on the viral isolate, with an N-terminal hydrophobic transmembrane anchor and a hydrophilic cytoplasmic C-terminal domain (Maldarelli *et al.*, 1993).

Two independent physiological activities of Vpu have been characterized. The first function of Vpu is the enhancement of the specific degradation of the HIV-1 receptor, CD4, in cell-free systems (Chen *et al.*, 1993) and *in vivo* (Willey *et al.*, 1992; Schubert and Strebel, 1994). Degradation of CD4 promotes the transport and subsequent processing of the HIV-1 envelope glycoprotein by releasing it from complexes with CD4 that trap both proteins in the endoplasmic reticulum (Kimura *et al.*, 1994). Direct interaction between Vpu and the cytoplasmic domain of CD4 is required, but not sufficient, for CD4 degradation (Bour *et al.*, 1995), and mutational analysis indicates that the hydrophilic cytoplasmic domain of Vpu is required for Vpu-mediated CD4 degradation (Schubert *et al.*, 1996). The second function of Vpu is to increase release of the virus particles (Terwilliger *et al*, 1989; Yao *et al.*, 1992). The effect of Vpu on virus particle release seems to be mediated from the post endoplasmic reticulum compartment (Schubert and Strebel, 1994), and the transmembrane domain of Vpu is sufficient for partial enhancement of virus release (Schubert *et al.*, 1996).

The identification of host-cell proteins that function in HIV replication has provided crucial insight into the complex life cycle of HIV-1. CD4 is known as the principal virus receptor (Dalgleish *et al.*, 1984), and chemokine receptors are also known as the co-receptors of HIV-1 (Alkhatib *et al.*, 1996; Feng *et al.*, 1996). Urokinase-type plasminogen activator is known for its involvement in the cleavage of HIV-1 gp120 during macrophage infection (Handley *et al.*, 1996), and cyclophilins are known to interact with HIV-1 Gag (Franke *et al.*, 1994; Thali *et al.*, 1994). Also, furin is known to cleave of HIV-1 gp160 into gp120 and gp41 (Hallenberger *et al.*, 1992). Recently, many other interacting proteins with the HIV-1 viral proteins have been identified using a yeast two-hybrid system. For the HIV-1 Vpu interacting protein, two proteins such as βTrCP and UBP were reported (Callahan *et al.*, 1988; Margottin *et al*, 1998). When we constructed the bait plasmid from HIV-1 Vpu for the yeast two-hybrid system, we found that the deletion mutant of HIV-1 Vpu had transactivation activity in the yeast system.

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Materials and Methods

Materials, media, strains All chemicals used for yeast transformation and β-galactosidase assays were purchased from Sigma (St. Louis, USA). Restriction enzymes, ligase, and Klenow fragment were obtained from Promega (Madison, USA). Synthetic dropout minimal (SD) medium with various supplements was used for yeast culture, maintenance, and selection of transformants (Ausubel *et al.*, 1995). Yeast strain EGY48 [*MATa, his3, trp1, ura3-52, leu::pLeu-LexAop6*/pSH18-34 (*LexAop-lacZ* reporter)] was used for the transformation of the bait plasmid.

Construction of deletion mutant plasmids For the construction of the bait plasmid, pEG202 containing the *His3* marker, yeast 2μ origin, *E. coli* pBR origin, and LexA DNA binding domain was used as a vector to express the LexA-Vpu fusion protein. The HIV-1 full-length Vpu cDNA, transmembrane deleted Vpu, helix domain I of Vpu, and helix domain II of Vpu were PCR-amplified from the HXB2 gene and cloned into the *Eco*RI-*Sal*I sites of pEG202. The primer pairs for full-length Vpu consisted of 5′-ccgaa ttcat gcaac ctata atagt agcaa tag-3′ and 5′-gccgt cgacc tacag atcat caata tccca-3′. For transmembane-deleted Vpu, the primer pairs consisted of 5′-gaatt cgaat atagg aaaat attaa gacaa-3′ and 5′-gtcga cctac agatc atcaa tatcc ca-3′. The primer pairs of the helix I domain were 5′-gaatt cgaat atagg aaaat attaa gacaa-3′ and 5′-gtcga cttag ccact gtctt ctgct ct-3′. For the helix II domain, the primer pairs were 5′-gaatt caatg agagt gaagg agaag ta-3′ and 5′-gccgt cgacc tacag atcat caata tccca-3′. The resulting wild-type and deletion mutant plasmids were named as pEGLexA-Vpu(Wt), pEGLexA-Vpu(ΔTM), pEGLexA-Vpu(H1), and pEGLexA-Vpu(H2), respectively.

Transactivation assay on yeast The EGY48 cells were transformed with plasmids containing LexA-Vpu wild-type or deletion mutants by the lithium acetate method, and were grown on synthetic agar medium (Ura⁻, His⁻) containing 2% glucose. Growing transformants were transferred to synthetic medium (Ura⁻, His⁻, Leu⁻) containing 2% glucose, and then incubated for 60 h at 30°C in an incubator. The growth on synthetic medium (Ura⁻, His⁻, Leu⁻) was determined for transctivation activity.

Quantitation of β-galactosidase activity Transformants containing each of the plasmids were cultured in the yeast synthetic media (Ura⁻, His⁻) containing 2% glucose until they reached a mid-log phase. Cell growth was monitored by the absorbance at 600 nm. The culture broth (0.2 ml) was taken and mixed with 0.7 ml of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, pH 7.0) containing 2-mercaptoethanol. Chloroform (50 μl) and 0.1% SDS (50 μl) were added to the mixture, and the cells were vortexed for 30 sec. The reaction substrate *o*-nitrophenyl β-D-galactopyranoside (ONPG) (0.16 ml) was added and the reaction was carried out at 30°C until a yellow color appeared. The reaction was stopped by the addition of 0.4 ml of 1 M Na_2CO_3. Cell debris was removed by centrifugation, and the absorbance of the supernatant was measured at 420 nm.

Results and Discussion

To find the HIV-1 Vpu interacting protein, we screened the T Jurkat cDNA library and found UBP to be an interacting protein, which was recently reported (Callahan *et al.*, 1998). To search for an interacting site between these two proteins, three deletion mutants were constructed based on structural differences, including mutant Vpu(ΔTM), helix I containing mutant Vpu(H1), and helix II containing mutant Vpu(H2), as shown in Fig. 1. All the transformants containing mutant plasmids were grown on synthetic medium (Ura⁻, His⁻). These growing transformants were transferred to a different synthetic medium (Ura⁻, His⁻, Leu⁻) and the transactivation of the *Leu2* reporter gene was determined by the growth on this medium. The mutants Vpu(ΔTM) and Vpu(H2) grew on the synthetic medium (Ura⁻, His⁻, Leu⁻), while wild-type Vpu and mutant Vpu(H1) could not (Fig. 2). This result showed that the mutant Vpu(ΔTM) and mutant Vpu(H2) had transactivation activity through protein-protein interaction. This was also confirmed by the ONPG test to quantify the β-galactosidase activity, which represented the

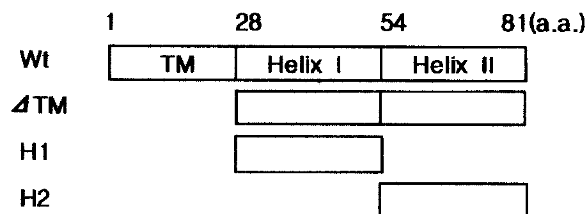

Fig. 1. Deletion mutants of HIV-1 Vpu. HIV-1 Vpu full-length cDNA was PCR-amplified and cloned into pEG202 to produce fusion proteins of LexA DBD with HIV-1 Vpu full protein (Wt), Vpu with deleted transmembrane domain (ΔTM), Vpu helix I domain (H1), and Vpu helix II domain (H2). Arabic numerals show the amino acid residues of the HIV-1 Vpu protein.

Fig. 2. Growth dependency on transactivation of the *Leu2* reporter gene. The transformations of EGY48 with pEGLexA-Vpu(Wt) (top-right in both A and B plate), pEGLexA-Vpu(ΔTM) (top-left), pEGLexA-Vpu(H1) (bottom-right), and pEGLexA-Vpu(H2) (bottom-left) were streaked on SD medium lacking uracil and histidine (plate A) and medium lacking uracil, histidine, and leucine (plate B). The plates were incubated at 30°C for 4 days under similar conditions.

transactivation of the *lacZ* reporter gene (Fig. 3). The transactivation activity of mutant Vpu(ΔTM) seemed to be caused by the H2 domain of Vpu, as the H1 domain has no transactivation activity. Despite this, the H1 domain could have some conformational effect since the activity of the H2 domain was lower than that of the ΔTM domain. It was quite interesting to compare the wild-type Vpu, which has no transactivation activity with the mutant Vpu(ΔTM), which showed high transactivation activity. This result suggested that the transmembrane domain of Vpu could block the interaction between Vpu and the transcriptional regulator protein.

When the amino acid sequences of H1 domain and H2 domain of Vpu were compared, we found that the H2 domain contained eight acidic amino acids and no basic amino acids (Fig. 4), whereas the H1 domain contained six acidic amino acids and eight basic amino acids. Many transcription factors contain an acidic domain which usually include glutamic acid-rich residues. The H2 domain of Vpu contained five glutamic acid residues like the acidic domain of some transcription factors, and this domain may cause the transactivation in the yeast system. HIV-1 Vpu interacts with CD4 in the endoplasmic reticulum and triggers CD4 degradation, presumably by proteasomes. During this process, Vpu may be cleaved by proteases and transferred to the nucleus with UBP, the Vpu-binding protein. UBP is a TPR-containing proteins, and many TPR-containing proteins are localized in the nucleus (Lamb *et al.*, 1995). Although it is still not clear on whether HIV-1 Vpu is involved in the transcription during virus infection, we propose that the transactivation may occur through this acidic H2 domain of HIV-1 Vpu.

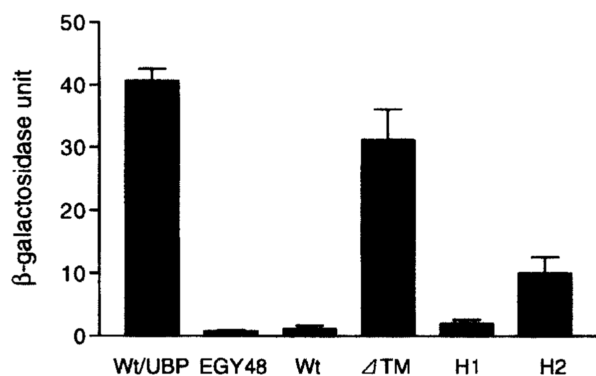

Fig. 3. Differential transactivation potentials of HIV-1 Vpu deletion mutants. The liquid β-galactosidase assay was performed in the transformants of yeast strain EGY48 producing fusion proteins of LexA-Vpu(Wt), LexA-(ΔTM), LexA-Vpu(H1), and LexA-Vpu(H2). Yeast cells containing wild-type Vpu and UBP, selected as a Vpu interacting protein, were used as a positive control. One unit of β-galactosidase activity was defined as the amount of enzyme required to hydrolyze 1 μmol of ONPG to *o*-nitrophenol and D-galactose per minute. The average values with standard errors of β-galactosidase activity are indicated based on three sets of independent experiments. Duplicate measurements for each sample were performed.

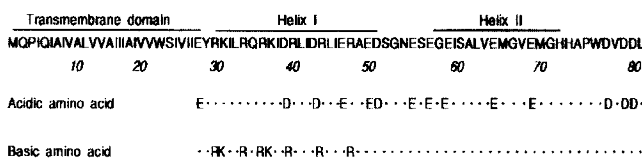

Fig. 4. Acidic and basic amino acids in the HIV-1 Vpu protein. The eighty-one amino acids are grouped into transmembrane domain, helix I domain, and helix II domain. Acidic and basic amino acids in the helix I and II domains are aligned.

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References

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M. and Berger, E. A. (1996) CC CKR5: a RANTES, MIP-1alpha, MIP-1 beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**, 1955–1958.

Ausubel, F., Brent, R., Kingston, R. E., Moore, D., Seidman, J. G., Smith, J. A. and Struhl, K. (1995) *Short Protocols in Molecular Biology*, 3rd ed., John Wiley & Sons, Inc., USA.

Bour, S., Schubert, U. and Strebel, K. (1995) The human immunodeficiency virus type 1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. *J. Virol.* **69**, 1510–1520.

Callahan, M. A., Handley, M. A., Lee, Y.-H., Talbot, K. J., Harper, J. W. and Panganiban, A. T. (1988) Functional interaction of human immunodeficiency virus type 1 Vpu and gag with a novel member of the tetratricopeptide repeat protein family. *J. Virol.* **72**, 5189–5197.

Chen, M. Y., Maldarelli, F., Karezewski, M. K., Willey, R. L. and Strebel, K. (1993) Human immunodeficiency virus type 1 Vpu protein induces degradation of CD4 *in vitro*: the cytoplasmic domain of CD4 contributes to Vpu sensitivity. *J. Virol.* **67**, 3877–3884.

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F. and Weiss, R. A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**, 763–767.

Feng, Y., Broder, C., Kennedy, P. E. and Berger, E. A. (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**, 872–877.

Franke, E. K., Yuan, H. E.-H. and Luban, J. (1994) Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* **372**, 359–362.

Friborg, J., Ladha, A., Gottlinger, H., Garzon, S., Haseltine, W. A. and Cohen, E. A. (1995) Functional analysis of the phosphorylation sites on the human immunodeficiency virus type 1 Vpu protein. *J. Acquired Immune Defic. Syndr.* **8**, 10–22.

Hallenberger, S., Bosch, V., Angliker, H., Shaw, E., Klenk, H. D. and Garten, W. (1992) Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. *Nature* **360**, 358–361.

Handley, M. A., Steigbigel, R. T. and Morrison, S. A. (1996) A role for urokinase-type plasminogen activator in human immunodeficiency virus type 1 infection of macrophages. *J. Virol.* **70**, 4451–4456.

Kimura, T., Nishikawa, M. and Ohyama, A. (1994) Intracellular membrane traffic of human immunodeficiency virus type 1 envelope glycoproteins: vpu liberates Golgi-targeted gp160 from CD4-dependent retention in the endoplasmic reticulum. *J. Biochem.* **115**, 1010–1020.

Klimkait, T., Strebel, K., Hoggan, M. D., Martin, M. A. and Orenstein, J. M. (1990) The human immunodeficiency virus type 1-specific protein vpu is required for efficient virus maturation and release. *J. Virol.* **64**, 621–629.

Lamb, J. R., Tugendreich, S. and Hieter, P. (1995) Tetratrico peptide repeat interactions: to TPR or not to TPR? *TIPS* **20**, 257–259.

Maldarelli, F., Chen, M. Y., Willey, R. L. and Strebel, K. (1993) Human immunodeficiency virus type 1 Vpu protein is an oligomeric type 1 integral membrane protein. *J. Virol.* **67**, 5056–5061.

Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas, D., Strebel, K. and Benarous, R. (1988) A novel human WD protein, h-bTrcp, that interacts with HIV-1 Vpu and connects CD4 to the ER degradation pathway through an F-box motiff. *Mol. Cell.* **1**, 565–574.

Schubert, U. and Strebel, K. (1994) Differential activities of the human immunodeficiency virus type 1-encoded Vpu protein are regulated by phosphorylation and occur in different cellular compartments. *J. Virol.* **68**, 2260–2271.

Schubert, U., Bour, S., Ferrer-Montiel, A. V., Montal, M., Maldarelli, F. and Strebel, K. (1996) The two biological activities of human immunodeficiency virus type 1 Vpu protein involve two separable structural domains. *J. Virol.* **70**, 809–819.

Strebel, K., Klimkait, T. and Martin, M. A. (1988) A novel gene of HIV-1, vpu, and its 16-kilodalton product. *Science* **241**, 1221–1223.

Strebel, K., Klimkait, T., Maldarelli, F. and Martin, M. A. (1989) Molecular and biochemical analyses of human immunodeficiency virus type 1 Vpu protein. *J. Virol.* **63**, 3784–3791.

Terwilliger, E. F., Cohen, E. A., Lu, Y. C., Sodroski, J. and Haseltine, W. A. (1989) Functional role of human immunodeficiency virus type 1 vpu. *Proc. Natl. Acad. Sci. USA* **86**, 5163–5167.

Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C. T., Sodroski, J. and Gottlinger, H. G. (1994) Functional association of cyclophilin A with HIV-1 virions. *Nature* **372**, 363–365.

Willey, R. L., Maldarelli, F., Martin, M. A. and Strebel, K. (1992) Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. *J. Virol.* **66**, 7193–7200.

Yao, X. J., Gottlinger, H., Haseltine, W. A. and Cohen, E. A. (1992) Envelope glycoprotein and CD4 independence of Vpu-facilitated human immunodeficiency virus type 1 cepsid export. *J. Virol.* **66**, 5119–5126.