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Characterization of twin-cysteine motif in the V2-loop region of gp120 in primate lentiviruses

Dane Bowder[a,c], Jesse Thompson[a,b], Kate Durst[a,c], Haley Hollingsead[a,c], Duoyi Hu[a,b], Wenzhong Wei[a,b], Shi-Hua Xiang[a,b,*]

[a] Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE 68583, United States
[b] School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, United States
[c] School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, United States

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ABSTRACT

The twin-cysteine motif (TCM) in the V2 loop region of gp120, identified in our previous report on the simian immunodeficiency virus mac239 (SIVmac239), is a conserved evolutionary element in all primate lentiviruses except for HIV-1 which has lost the TCM during cross-species transmission. In this study, we have further explored the TCM in other SIV and HIV-2 strains. Our data shows that strains from different evolutionary lineages have different phenotypes when the twin-cysteines are removed. In the SIVsm/HIV-2 lineage, removal of the twin-cysteines decreases envelope trimer stability, but in the SIVagm lineage, a blockage of gp160 processing is observed. Molecular modeling has confirmed that the twin-cysteines do form a disulfide bond in the gp120 subunit, which interacts with the V1 loop to stabilize the envelope trimer. Therefore, we hypothesize that if the TCM is added back to HIV-1, it will enhance envelope stability for vaccine immunogen design.

1. Introduction

Primate lentiviruses (PLV) are known to include the simian immunodeficiency viruses (SIVs) along with the human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) which have posed a major threat to human health globally in the 21st century (Centers for Disease, C., Prevention, 2006; Dore, 2000; Hahn et al., 2000; Locatelli and Peeters, 2012). It is known that the human immunodeficiency viruses (HIV-1 and HIV-2) entered the human population through cross-species transmissions of SIVs around the turn of the twentieth century (Compton et al., 2013; Gao et al., 1999; Hillis, 2000; Sharp and Hahn, 2011; Takehisa et al., 2009). Four independent transmission events have given rise to HIV-1 groups M, N, O and P (Sharp and Hahn, 2011). A separate lentiviral lineage, HIV-2, emerged in humans at least eight times via cross-species transmissions from simian immunodeficiency viruses of sooty mangabey monkeys (SIVsm) (Gao et al., 1992; Hirsch et al., 1995). There are at least forty SIV species found in African monkeys which have a great potential to continually jump to humans (Sharp and Hahn, 2010; Sharp et al., 2013). Therefore, it is very important to understand PLV pathogenesis, evolution and transmission. During the past three decades, tremendous efforts have been made in the fight against the HIV-1 major group M epidemic. Particularly, there has been extensive effort to understand the envelope spike, which is the sole viral protein on the surface of HIV virions and plays a critical role in viral infection, evolution and transmission. The envelope spike has been known to have a trimeric structure for decades, but the structure of the envelope trimer was not solved until 2013 (Julien et al., 2013; Lyumkis et al., 2013). According to the envelope trimer structures reported, the major loops V1, V2 and V3 of gp120 are packed into the apex of envelope trimer, which is essential for the loops contribution to trimeric stabilization (Do Kwon et al., 2015; Julien et al., 2013; Lyumkis et al., 2013; Pancera et al., 2014). Another important finding from vaccine clinical trials is that the V2 loop has been found to be an important immunogen in the RV144 vaccine trial by inducing neutralizing antibodies (de Souza et al., 2012; Karasavvas et al., 2012; Kim et al., 2015; van Gils et al., 2011). Previously, we reported that the twin-cysteine motif (TCM) in the V2 loop region of gp120 in the SIVmac239 strain plays an important role in the stabilization of the envelope trimer (Bohl et al., 2013). Substitution of the cysteines singly or doubly causes drastic gp120 shedding and leads to reduced viral infection. Based on envelope sequence alignment of primate lentiviruses, the twin-cysteines are present in all SIV and HIV-2 strains but not in HIV-1 strains. More interestingly, in SIVcpz, some strains have the TCM, such as SIVcpzANT, while others do not, such as SIVcpzTAN. According to the PLVs evolutionary relationship, it is apparent that the SIVcpz strains are close to HIV-1 which do not contain the TCM. This strongly suggests that the TCM

* Corresponding author at: Nebraska Center for Virology, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Morrison Center 143, 4240 Fair Street, Lincoln, NE 68583-0900, United States.
E-mail address: sxiang2@unl.edu (S.-H. Xiang).

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was lost in the SIVcpz and HIV-1 strains during the viral evolution process. However, in the SIVsm, SIVmac and HIV-2 lineages, the TCM is maintained. This raises a very interesting question about the importance of the TCM in PLVs and whether this TCM is associated with HIV pathogenesis or transmission. Thus, in this project, we have studied the role of the TCM in different SIV isolates and in HIV-2, in which the twin-cysteines are preserved. To further support our hypothesis we have also introduced the twin-cysteines back to HIV-1 strains to observe the effect of the TCM in HIV-1 strains, which is significant for envelope based vaccine immunogen design.

2. Results

2.1. Removal of the twin-cysteine motif from HIV-2 ST results in a decrease in envelope stability

In our previous study, the SIVmac239 virus showed a clear increase in gp120 shedding when either one or both of the twin cysteines were substituted with alanine residues (Bohl et al., 2013). HIV-2, like SIVmac, is originally derived from SIVsmm, but unlike HIV-1, the twin-cysteine motif is preserved in HIV-2 through this cross-species transmission (Fig. 1). Therefore, it is believed that the twin-cysteine motif in HIV-2 strains should play the same role as in SIVmac strains, such as SIVmac239. To test this hypothesis, we removed the cysteines by substitution with alanine residues individually or together from the envelope of the HIV-2 ST strain. The gp120 shedding assay was then performed for these mutants, and a clear increase in envelope shedding was seen, as well as a drastic decrease in the gp120/gp41 association index (Fig. 2 and Table 1). This decrease in envelope stability correlated well with a decrease in envelope function, evidenced by the effect on membrane fusion capability, which dropped to the level of background (Fig. 2B and Table 1). This indicates that the twin-cysteines in the HIV-2 ST strain play a role in the stability of the envelope trimer similar to that of SIVmac239. These results demonstrate that the twin-cysteine motif functions in HIV-2 to stabilize the Env trimer. We have also looked at the envelope processing and calculated the processing index for each mutant (Table 1). The cysteine mutants of HIV-2 ST appeared to be significantly affected.

2.2. Alteration to the twin-cysteine motif changes the envelope processing in SIVagm

The SIVagm lineage is a separate lineage which is closely related to SIVsyk (Courgnaud et al., 2001). In this lineage, the twin-cysteine motif is also present in all SIVagm strains. We sought to determine whether the twin-cysteines also play a role in the stabilization of the envelope trimer in the viral strains in the older lineages of PLVs. The strain SIVagmTan1 was chosen for this test. The twin-cysteines of SIVagmTan1 were removed by substituting the cysteine residues with alanine residues, both singly and doubly. The gp120 shedding assay was then performed with these mutants, but an unexpected phenotype was observed. A clear accumulation of gp160 with less gp120 in the cell lysate fraction occurred, along with a complete absence of gp120 found in the supernatant (Fig. 3A and Table 1).

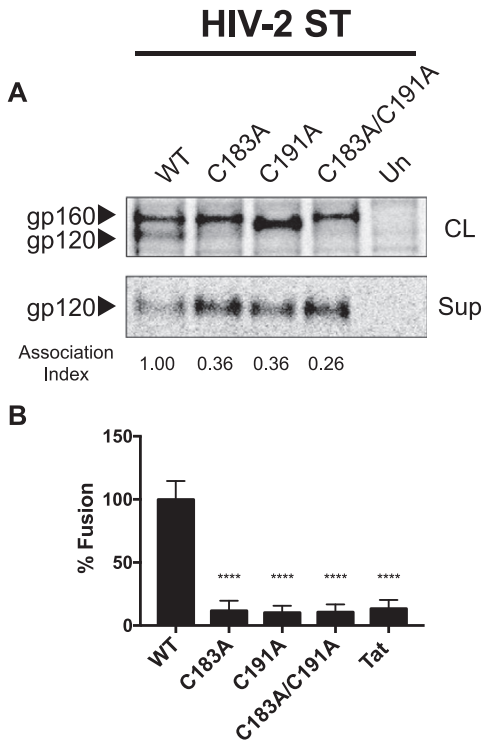

Fig. 2. Characterization of the Twin-cysteine motif (TCM) in HIV-2. **(A).** gp120 shedding assay of twin-cysteine substitution mutants from HIV-2 ST strain. WT, wild-type, single substitution, C183A or C191A, or double substitutions, C183A/C191A. The association index was calculated as described in the Methods, and is shown below the shedding gel, values are representative of the average of three separate experiments. **(B).** Cell-cell fusion assay of the same mutants as in panel A. Values are representative of three separate experiments performed in quadruplicate. Significance was determined by Student's T-test ($p \leq 0.05$).

This result suggests that the mutations caused a change in envelope processing, as it appears that no gp160 is being cleaved into gp120 and gp41. This suggests that removal of the TCM changed the envelope structural conformation such that it is not accessible for cleavage by the furin protease. When these mutants were used in a fusion assay, there were significant reductions in their membrane fusion activities (Fig. 3B and Table 1). The same fusion results were also seen in another African green monkey SIV strain, SIVagm155 (Fig. 3C and Table 1).

2.3. Impacts of adding back the twin-cysteine motif to HIV-1

To test our hypothesis that the twin-cysteine motif was evolutionarily lost during the transmission from SIV to HIV-1, we hypothesized that if the twin cysteine motif was added back to HIV-1 strains, trimer stability would improve. According to the sequence alignment of the twin-cysteine region, the proline at position 183 (P183) is well conserved and is aligned with the

Species	strain	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196
HIV-1	HXBc2	C	S	F	N	I	S	T	S	I	R	G	K	V	Q	K	E	Y	A	F	F	Y	K	L	D	V	V	P	I	D	·	N	N	·	·	·	·	·	T	S	Y	R	L	I	S	C
	JR-FL	C	S	F	N	I	T	T	S	I	R	D	E	V	Q	K	E	Y	A	L	F	Y	K	L	D	V	V	P	I	D	·	N	N	·	N	·	·	T	S	Y	R	L	I	S	C
	ADA	C	S	F	N	I	T	T	S	I	R	D	K	V	K	K	D	Y	A	L	F	Y	R	L	D	V	V	P	I	D	·	N	D	·	N	·	·	T	S	Y	R	L	I	N	C
HIV-2	ST	C	Q	F	N	M	T	G	L	E	R	D	K	·	K	K	L	Y	E	T	W	Y	S	K	D	V	V	C	E	·	S	N	D	T	K	K	E	K	T	C	Y	·	M	N	H	C
SIV	mac239	C	K	F	N	M	T	G	L	K	R	D	K	·	K	K	E	Y	E	T	W	Y	S	A	D	L	V	C	E	Q	G	N	N	T	G	N	E	S	R	C	Y	·	M	N	H	C
	agmTan1	C	S	F	A	M	M	G	Y	R	R	D	·	V	K	K	K	Y	S	T	W	Y	D	Q	E	L	V	C	E	K	E	N	N	T	T	G	T	R	G	C	Y	·	M	I	H	C
	agm155	C	T	F	A	M	A	G	Y	I	R	D	Q	·	K	K	N	Y	V	V	W	N	D	A	E	I	F	C	K	R	S	T	S	H	N	G	T	K	E	C	Y	·	M	I	H	C

Twin-Cysteine Motif

Fig. 1. The gp120 V2 loop sequence alignment of HIV-1, HIV-2 and SIV species used in this study. The twin-cysteine motif (TCM) in SIV and HIV-2 species are labeled. The sequence numbering is based on the HIV-1 HXBc2 standard sequence (Smith et al., 2014).

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