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In vitro and *in silico* binding study of the peptide derived from HIV-1 CA-CTD and LysRS as a potential HIV-1 blocking site

Poonsap Na Nakorn [a], Witcha Treesuwan [d], Kiattawee Choowongkomon [a], Supa Hannongbua [b], Nonlawat Boonyalai [a,c,*]

[a] Department of Biochemistry, Faculty of Science, Kasetsart University, Jatujak, Bangkok 10900, Thailand
[b] Department of Chemistry, Faculty of Science, Kasetsart University, Jatujak, Bangkok 10900, Thailand
[c] Center of Excellence for Innovation in Chemistry, Faculty of Science, Kasetsart University, Jatujak, Bangkok 10900, Thailand
[d] Institute of Food Research and Product Development, Kasetsart University, Jatujak, Bangkok 10900, Thailand

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ABSTRACT

The assembly process in HIV-1 has become a new target for infected HIV-1 patient treatment. During this process, the viral genomic RNA and precursor protein are assembled at the permeable membrane and $tRNA^{Lys3}$ is packed into a new virion as the primer for the reverse transcription process. The packaging of $tRNA^{Lys3}$ arises from the interaction of HIV-1 Gag and hLysRS. To better understand the formation of this ternary complex, the interaction study of LysRS–peptide complex using a combination of circular dichroism, molecular dockings and molecular dynamic simulations are reported here. The circular dichroism experiments confirm that the sh-H4 peptide, containing 10 amino acid residues from helix4 of C-terminal domain of HIV-1 capsid protein (CA-CTD), can be induced to form a helical structure upon binding to hLysRS. Molecular docking analysis of LysRS (hLysRS and eLysRS) with the sh-H4 peptide revealed the two possible arrangements of the peptide upon the binding event. Molecular dynamics based free energy calculations of the peptide binding process are used to determine the interactions as well as the important amino acid residues involving in binding. The peptide is found to lie against helix 7 of LysRS in a perpendicular fashion. Additionally, the peptide preferably interacts with hLysRS over eLysRS including strong hydrogen bond interactions between R247-Q219 and R241-E212. Interestingly, these amino acid residues are found in both LysRS and CA-CTD. These important residues appear to be a vital feature of the LysRS-CA-CTD complex and may ultimately lead to the inhibitor design to block the Gag–LysRS interaction.

1. Introduction

HIV-1 has been under intense investigations for more than 20 years. Over 40 million people are currently living with HIV infection, which is the cause of Acquired Immunodeficiency Syndrome (AIDS) (Turner and Summers, 1999). In the past few years, a great deal of progress has been made towards understanding the detailed biochemical function of each viral component in order to find ways to reduce the mortality and morbidity related to HIV-1 infection. Currently, the therapy for HIV-1 infection relies on HIV-1 reverse transcriptase inhibitors; such as etravirine (INTELENCE^{TM}), efavirenz and viramune (Nevirapine) for non-nucleotide reverse transcriptase inhibitor (NNRTI) (Das et al., 2004; Hargrave et al., 1991; James, 1997), zidovudine (AZT), Zerit (Stavudin) and teuofovir disoproxil fumarate (Viread) for nucleotide reverse transcriptase inhibitor (NRTI) (Emau et al., 2006; Fischl et al., 1987; Horwitz et al., 1966), and HIV protease inhibitors such as darunavir (PREZIST tablets), ritonavir (Norvir) and nelfinavir (viracept) (De Meyer et al., 2005; Pakker et al., 1997; Zhang et al., 2001) while enfuvirtide inhibits gp120 in the fusion process of HIV-1 and host (Petteway et al., 1994). Although these drugs show a clinical benefit, their efficiency is reduced by viral mutations (Schinazi et al., 1996). Therefore, new targets and inhibitors for the inactivation of the virus life cycle are required. One of the new anti-HIV-1 targets is HIV-1 integrase. Current integrase inhibitors (raltegravir and elvitegravir) are in pre-clinical research (Beck-Engeser et al., 2009). Raltegravir was found to inhibit the replications of murine leukemia virus (Beck-Engeser et al., 2009). The results revealed that raltegravir could induce the development of auto-antibodies in infected mice 3 times faster than in untreated mice. Recent additional anti-HIV-1 targets include the disruption of the capsid formation (Mateu, 2009) as well as the inhibition of the $tRNA^{Lys3}$ packaging complex forming (Kleiman et al., 2010).

Assembly is the last step of HIV-1 life cycle before the budding of the virus. The viral genomic RNA, Gag, Gag-Pol, $tRNA^{Lys3}$ and

Abbreviations: HIV-1, human immunodeficiency virus type I; CA-CTD, C-terminal domain of capsid protein; sh-H4, short helix4 of HIV-1 CA-CTD (amino acids 211–220); eLysRS, *E.coli* Lysyl-tRNA synthetase; hLysRS, human Lysyl-tRNA synthetase; MD, molecular dynamics

* Corresponding author.
E-mail address: nonlawat.b@ku.ac.th (N. Boonyalai).

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hLysRS are packaged into a new virion, called the $tRNA^{Lys3}$ packaging complex (Kleiman and Cen, 2004). It is noteworthy that among three $tRNA^{Lys}$ isoacceptors namely $tRNA^{Lys1}$, $tRNA^{Lys2}$ and $tRNA^{Lys3}$, only $tRNA^{Lys3}$ is used as the primer for the reverse transcription (Mak et al., 1994). The $tRNA^{Lys3}$ is selected for packaging into new viral particles by the signal from hLysRS (Kleiman and Cen, 2004). *In vitro* GST pull-down assays revealed that the interaction of Gag and hLysRS is dependent upon the last 54 amino acids of the capsid C-terminal domain (CA-CTD) of Gag and amino acids 208–259 of hLysRS, the consensus sequence known as motif 1 in the class II of amino acyl-tRNA synthetase (Javanbakht et al., 2003). The model formation of the $tRNA^{Lys3}$ packaging complex was also studied and reviewed in 2004 (Kleiman and Cen, 2004). The complex is formed when Gag/Gag-Pol/viral RNA complex interacts with a $tRNA^{Lys3}$/hLysRS complex. The Gag interacts with hLysRS while Gag-Pol interacts with $tRNA^{Lys3}$ (Kleiman and Cen, 2004). Binding interactions between Gag and hLysRS were characterized by Kovaleski and co-workers (Kovaleski et al., 2006), indicating the equilibrium binding constant of 310 ± 80 nM and that only the capsid domain of Gag can sufficiently bind to hLysRS with the same affinity as the full length Gag. Additionally, the complex between hLysRS and CA-CTD was proposed to be a heterodimeric complex (Kovaleski et al., 2006). The important amino acid residues involved in this interaction were also mapped (Kovaleski et al., 2007). Only helix 4 of CA-CTD binds to hLysRS. The amino acids Glu212, Met214, Met215, Thr216 and His 226 are the important residues on CA-CTD. Molecular docking on LysRS and CA-CTD was also performed but eLysRS was used as a template due to the lack of the three-dimensional structure of hLysRS. However, the structure of hLysRS was finally solved at 2.3 Å resolutions (Guo et al., 2008). Interestingly, hLysRS forms a tetramer (Fig. 1) by using two symmetric dimers to form an asymmetric tetramer (hLysRS-*ab* and hLysRS-*cd*). The bottom side (hLysRS-*b* and hLysRS-*d*) functions as "on" interacting tetramer interface while the top side (hLysRS-*a* and hLysRS-*c*) functions as "off" interacting tetramer interface with a 5 Å gap. The couple equilibrium between monomer-dimer and dimer-tetramer was determined using fluorescence anisotropy and the fluorescence quantum yield (FQY). The dissociation constants (K_ds) of monomer-dimer and dimer-tetramer equilibrium were 2 and 280 nM, respectively. Additionally, gel filtration has shown that there are the presence of two species, dimer and tetramer. It is therefore concluded that there is a dynamic dimer-tetramer oligomerization for hLysRS and hLysRS is a loose tetramer in solution. Since the structure of hLysRS is available, it is now convenient to ascertain a better understanding on the formation of the hLysRS–CA-CTD ternary complex.

In this study, the combination of experimental and computational methods suggests that only 10 amino acid residues of helix 4 of CA-CTD, called sh-H4 peptide (Fig. 1C) are vital for the interaction with LysRS. We use circular dichroism to demonstrate the importance of the critical length of the peptide. Molecular docking and molecular dynamic simulation are used to reveal the specific arrangement of the peptide and elucidate the crucial amino acid residues on LysRS in this interaction. This work clearly opens the way for further developments in the design of HIV-1 peptide inhibitors.

2. Methodology

2.1. Protein overexpression and purification

The plasmid pM368 (Shiba et al., 1997) was transformed into *E.coli* BL21 (DE3) competent cell by electroporation and selected by ampicillin resistance. One colony was inoculated in Luria-Bertani (LB) medium at 37 °C overnight. The culture was transferred to 1 liter of fresh LB medium containing 100 μg/ml ampicillin. Cells were then incubated in the orbital shaker 220 RPM at 37 °C. When the OD_{600} reached approximately 0.6, cells were induced by adding isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 1 mM, and further incubated for 3 h. The cells were harvested and the pellet was resuspended in extraction buffer (50 mM Tris–Cl pH 8.0, 0.5 M sodium chloride and 5 mM immidazole), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM β-mercaptoethanol (β-ME) and 1% Triton X-100. After the cells were disrupted using a sonicator, the histidine-tagged hLysRS was purified over a Ni^{2+}-affinity column using an FPLC. Bound protein was eluted by application of a linear gradient of 30–500 mM imidazole in elution buffer (50 mM Tris–Cl pH 8, 0.5 M sodium chloride and 0.5 M imidazole). The purity of recombinant protein was confirmed by 12% SDS–PAGE with the expected molecular weight of 68 kDa for monomer hLysRS. The fractions containing hLysRS were pooled, dialyzed overnight against dialysis buffer (50 mM Tris–Cl, pH 8.0 and 100 mM sodium chloride) and concentrated using a 30 kDa molecular weight cutoff concentrator. Proteins were flash frozen and stored at −80 °C until used.

2.2. CD spectroscopy

All CD studies were performed on a J-715 spectropolarimeter (Jasco, Japan) using a quartz cuvette with a path length of 0.1 mm, 1 nm bandwidth and a response time 4 s for far-UV CD measurements. Two peptides called wh-H4 and sh-H4 were purchased from Genemed Synthesis Inc (USA). According to HPLC, the purity of peptides was 98% and 95% for wh-H4 and sh-H4 peptide,

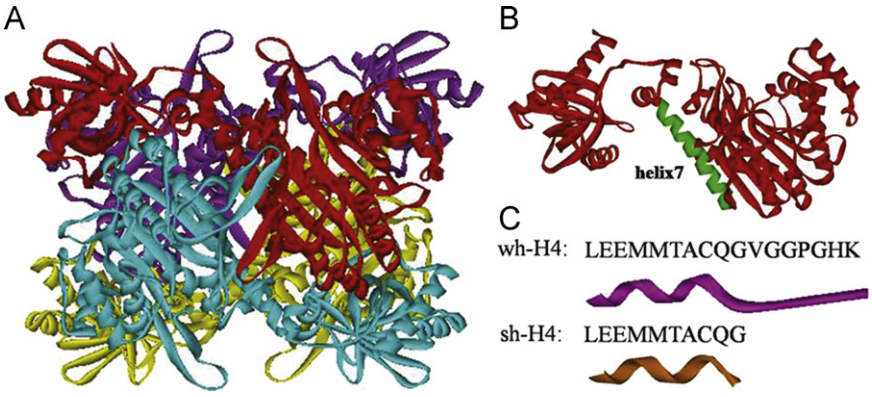

Fig. 1. The schematic structure of hLysRS, wh-H4 and sh-H4 peptide. (A) Tetrameric form of hLysRS; red is chain *a*, cyan is chain *b*, purple is chain *c* and yellow is chain *d*. (B) Monomeric form of hLysRS. The helix 7 is highlighted in green. (C) The wh-H4 peptide contains 17 amino acid residues while the sh-H4 contains 10 amino acid residues. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)