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Structural insights into the stabilization of the human immunodeficiency virus type 1 capsid protein by the cyclophilin-binding domain and implications on the virus cycle

Juliana R. Cortines [a,1,2], Luís Mauricio T.R. Lima [b,3], Ronaldo Mohana-Borges [c,4], Thiago de A. Millen [a,2], Luciane Pinto Gaspar [a,2,5], Jason K. Lanman [d,6,7], Peter E. Prevelige Jr. [d,7], Jerson L. Silva [a,*]

[a] Instituto de Bioquímica Médica, Instituto Nacional de Biologia Estrutural e Bioimagem, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil
[b] School of Pharmacy, Federal University of Rio de Janeiro—UFRJ, Rio de Janeiro 21941-590, Brazil
[c] Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil
[d] Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

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ABSTRACT

During infection, human immunodeficiency virus type 1 (HIV-1) interacts with the cellular host factor cyclophilin A (CypA) through residues 85–93 of the N-terminal domain of HIV-1's capsid protein (CA). The role of the CA:CypA interaction is still unclear. Previous studies showed that a CypA-binding loop mutant, Δ87–97, has increased ability to assemble *in vitro*. We used this mutant to infer whether the CypA-binding region has an overall effect on CA stability, as measured by pressure and chemical perturbation. We built a SAXS-based envelope model for the dimer of both WT and Δ87–97. A new conformational arrangement of the dimers is described, showing the structural plasticity that CA can adopt. In protein folding studies, the deletion of the loop drastically reduces CA stability, as assayed by high hydrostatic pressure and urea. We hypothesize that the deletion promotes a rearrangement of helix 4, which may enhance the heterotypic interaction between the N- and C-terminal domains of CA dimers. In addition, we propose that the cyclophilin-binding loop may modulate capsid assembly during infection, either in the cytoplasm or near the nucleus by binding to the nuclear protein Nup385.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is a single-stranded RNA enveloped virus from the *Retroviridae* family. HIV-1 is the etiological agent of the human acquired immunodeficiency syndrome (AIDS). The primary target cells in human hosts are the CD4+ cells. The HIV life cycle consists of virus binding, fusion, capsid uncoating and genome integration in the host cell, and it culminates with progeny production and consequent budding of immature, spherical and non-infectious viruses.

The virally encoded protease cleaves viral Gag polyprotein into independent structural proteins, a process that is common to all retroviruses. These proteins, capsid (CA), matrix (MA) and nucleocapsid (NC) form the viral capsid, underlie the lipid bilayer and associate with RNA, respectively, forming a mature particle with the viral core becoming centralized, with predominantly fullerene-cone shaped cores [1]. Approximately 1500 copies of CA molecules arranged as hexameric subunits and compose the viral capsid body [2–4]. The introduction of 12 pentamers at the extremities of the core guarantees its cone-shaped morphology [4–7]. The hexamers are built by CA, where the N-terminal domain (NTD) forms the outer ring, and the C-terminal domain (CTD) constitutes the inner lobes of the subunit. Homotypic (CTD–CTD and NTD–NTD) and heterotypic (NTD–CTD) interactions maintain the hexameric arrangement. However, only the CTD–CTD interaction can be observed in solution in unassembled CA [2,7–10].

The NTD is linked to the CTD via a 5-residue flexible linker (amino acids 146–150), with a "hinge" function that likely contributes to the HIV-1 pleomorphism and the ability of CA to form both hexamers and pentamers [4,7,11,12]. The arrowhead-shaped NTD is formed by 6 α-helices, a 3_{10} helix and an anti-parallel β-hairpin, with a mass of ~17 kDa. The β-hairpin is formed upon proteolytic cleavage during maturation and plays an important role in stabilizing the mature capsid

* Corresponding author. Tel.: +55 21 3938 6756.
E-mail addresses: cortines@micro.ufrj.br (J.R. Cortines), mauricio@pharma.ufrj.br (L.M.T.R. Lima), mohana@biof.ufrj.br (R. Mohana-Borges), thiagomillen@yahoo.com.br (T.A. Millen), lgaspar@bio.fiocruz.br (L.P. Gaspar), jlanman@purdue.edu (J.K. Lanman), prevelig@uab.edu (P.E. Prevelige), jerson@bioqmed.ufrj.br (J.L. Silva).
[1] Instituto de Microbiologia Paulo de Góes, Departamento de Virologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil.
[2] Tel.: +55 21 3938 6756.
[3] Tel.: +55 21 3938 6639.
[4] Tel.: +55 21 3938 6538.
[5] Programa de Vacinas Virais, Instituto de Tecnologia em Imunobiológicos, Fundação Oswaldo Cruz, Rio de Janeiro, RJ 21045-900, Brazil.
[6] Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA.
[7] Tel.: +1 205 975 5327.

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protein conformation [13–15]. Mutational studies demonstrated that the N-terminal region is likely involved in the regulation of the viral capsid morphology [14,16]. Composed of 4 α-helices, the CTD has an overall globular structure, with a mass of ~8 kDa. The CTD is responsible for the oligomerization of the Gag polyprotein and the dimerization of the mature capsid protein. The dimerization domain involves helices 8 and 9, where Trp 184 is the major contributor to the hydrophobicity of the dimer interface [17].

During the late phase, host cyclophilin A (CypA), from the peptidyl-prolil isomerase family, is incorporated into nascent virions in a 1:10 ratio and binds to the N-terminal domain of CA at the cyclophilin-binding loop (residues 85–93). CypA catalyzes the isomerization of the Gly89 – Pro90 bond, which suggests that the immunophilin may act as a chaperone during the HIV-1 life cycle. Interestingly, it was observed that the CA:CypA interaction affects the dimerization of CA, thus suggesting a long-range effect [18–22].

Cyclophilin is necessary for steps during the early phase, before reverse transcription [20] and could be involved, paradoxically, in either viral capsid assembly or disassembly [18,21,22]. A growing amount of evidence also shows that CypA may modulate HIV-1's sensitivity to host factors since it was found to be essential for the TRIM5α restriction of HIV-1 infection in Old World monkeys [23]. A crucial step in any viral life cycle is capsid uncoating. It was thought that HIV-1 readily uncoats in the cytoplasm. However, recent data showed intact HIV capsids at the nucleus pore and the nuclear protein Nup358 is directly involved in HIV-1 CA interaction, capsid nuclear localization and pre-integration complex nuclear entry. Nup358 has a cyclophilin moiety that binds to CA with higher affinity than the cytoplasmatic CypA [24,25].

To understand the role of cyclophilin binding on the capsid protein, Ganser-Pornillos *et al.* constructed a mutation, Δ 87–97, that inhibited the CA:CypA interaction [6]. It consisted of a deletion on the NTD cyclophilin binding loop between helices 4 and 5 and a replacement by two glycines (Fig. 1). This mutant showed an enhanced capacity to form tubes *in vitro* in comparison with the wild-type CA. These results suggest that the CA:CypA interaction may inhibit off-pathway aggregation, thus allowing the formation of a longer, organized tubular structure [6,18,22].

In the present study, we find that the cyclophilin binding loop region is necessary for HIV-1 capsid protein stability and plays a role in the maintenance of capsid integrity and assembly during infection. We also show a new dimer conformation for the capsid protein in solution.

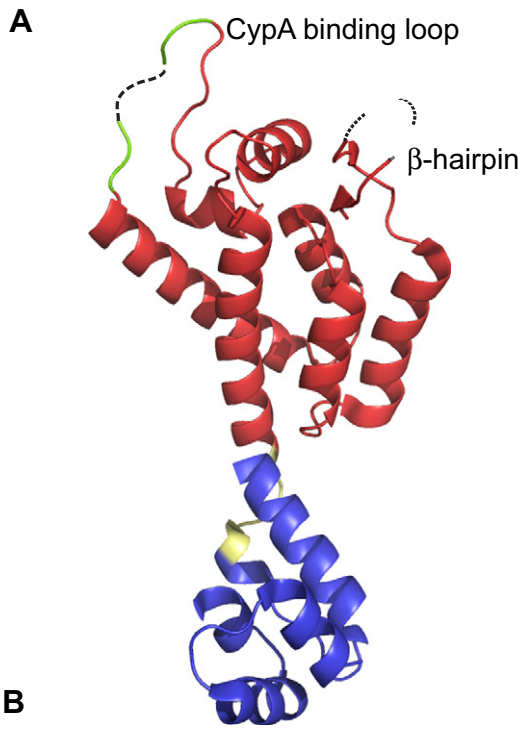

Fig. 1. *HIV capsid protein.* **(A)** The N-terminal domain (NTD) comprises helices 1–7 (red, residues 1–146). The cyclophilin binding loop colored green (residues 85–93). The linker region is colored yellow (residues 146–150). The C-terminal domain (CTD) is represented by helices 8–12 (blue, residues 151–231). **(B)** The primary sequence of the wild-type cyclophilin-binding loop and of the mutated region underlined in wild type. Also shown is the primary sequence of the mutated loop, substituted by two glycines highlighted in bold (GG). Dashed lines in the cyclophilin binding loop and in the β-hairpin represent the missing densities from the crystal structure. Residues 176–187 are also missing from the original structure. Numbers represent the position of the first and last residues in the capsid protein sequence. Figure was created using PyMOL (DeLano Scientific LLC, San Carlos, CA, USA) using PDB entry 3H47.

2. Material and methods

2.1. Chemicals

All reagents were of analytical grade. Distilled water was filtered and deionized through a Millipore water-purification system. Ultrapure urea and Tris were purchased from Sigma-Aldrich (St. Louis, MO). The temperature throughout all fluorescence experiments was controlled by a water-ethylene glycol bath at 25 °C ± 1 °C. Tris was the buffer of choice because the pH does not vary significantly under high pressures.

2.2. Protein purification

The protocol for capsid protein purification is detailed elsewhere [26]. For the mutant, a 30% *w/v* during the ammonium sulfate precipitation was used. All other steps were identical for both purifications. Protein concentration was determined using the extinction coefficient at 280 nm of 33,460 M^{-1} cm^{-1} for both proteins (http://web.expasy.org/protparam/).

2.3. Small angle X-ray scattering measurements and data analysis

Small angle X-ray scattering (SAXS) was performed at 25 °C using a Gabriel-type one-dimensional position-sensitive detector (PSD 1D Hecus) in the D11-SAS1 beam line on the National Synchrotron Light Laboratory, Campinas, SP, Brazil. Measurements were performed with a wavelength of 1.488 Å and a sample-detector distance providing a q-range from 0.02 $Å^{-1}$ to 0.25 $Å^{-1}$ [27], where q is the modulus of the scattering vector (calculated according to $q = (4\pi/\lambda)\sin\theta$, where λ is the wavelength used and 2θ is the scattering angle). The scattering curves of the protein solutions and buffers were collected in several successive frames of 900 s each. The data reduction included the normalization of the one-dimensional scattering profile to the intensity of transmitted incident beam, detector response, incident beam intensity, sample absorption and concentration and blank subtraction.

Measurements were performed at protein concentrations of 4.5 mg/mL and 3.6 mg/mL (175 μM and 146 μM) diluted in Tris–HCl 10 mM, pH 8.0 for the WT and mutant proteins, respectively, at which condition both proteins are dimeric in solution. Monodispersity and radius of gyration (R_g) of the specimen were evaluated using the linearity of the Guinier plot. The oligomeric state for the proteins in solution was confirmed using lysozyme as the standard. The R_g and the scattered intensity, $I_0(q)$, were inferred, respectively, from the slope and the intercept of the linear fit of $\ln[I(q)]$ versus q^2 in the q-range $q \times R_g < 1.3$. The maximum dimension, D_{max}, for the proteins in solution was estimated from the distance distribution function $p(r)$ using GNOM [28].

The resolution of the data was determined by:

$$R = 2\pi/q_{max} \quad (1)$$