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Crystal Structure of Human Cyclophilin A Bound to the Amino-Terminal Domain of HIV-1 Capsid

Theresa R. Gamble,* Felix F. Vajdos,* Sanghee Yoo,* David K. Worthylake, Megan Houseweart, Wesley I. Sundquist, and Christopher P. Hill
Biochemistry Department
University of Utah
Salt Lake City, Utah 84103

Summary

The HIV-1 capsid protein forms the conical core structure at the center of the mature virion. Capsid also binds the human peptidyl prolyl isomerase, cyclophilin A, thereby packaging the enzyme into the virion. Cyclophilin A subsequently performs an essential function in HIV-1 replication, possibly helping to disassemble the capsid core upon infection. We report the 2.36 Å crystal structure of the N-terminal domain of HIV-1 capsid (residues 1–151) in complex with human cyclophilin A. A single exposed capsid loop (residues 85–93) binds in the enzyme's active site, and Pro-90 adopts an unprecedented *trans* conformation. The structure suggests how cyclophilin A can act as a sequence-specific binding protein and a nonspecific prolyl isomerase. In the crystal lattice, capsid molecules assemble into continuous planar strips. Side by side association of these strips may allow capsid to form the surface of the viral core. Cyclophilin A could then function by weakening the association between capsid strips, thereby promoting disassembly of the viral core.

Introduction

The viral Gag protein and its proteolytically derived maturation products serve as the major structural components of the HIV-1 virion (Gelderblom et al., 1992; Hunter, 1994). Late in the infectious cycle, Gag accumulates at the cell membrane and assembles into immature virions that bud from the cell. Gag plays the central role in this assembly process and can form budded, virus-like particles in the absence of any other viral protein. Gag is also responsible for packaging the other essential components of the virion, either as additional domains of the frame-shifted Gag-Pol polyprotein (i.e., protease, reverse transcriptase, and integrase) or through noncovalent binding interactions (e.g., RNA, envelope, and viral protein R).

As the immature virion buds, the membrane-bound Gag protein is cleaved by the viral protease into three new proteins: matrix (MA; 132 residues), capsid (CA; 230 residues), and nucleocapsid (NC; 54 residues), as well as three smaller polypeptides. The processed proteins subsequently undergo a dramatic morphological rearrangement, termed "maturation," to create the infectious viral particle. Upon maturation, the MA protein lines the inner surface of the viral membrane while the CA protein forms a distinctive conical core structure that encloses the NC/RNA complex at the center of the virion. The viral particle must subsequently disassemble or rearrange when the virus infects a new cell, in order to allow reverse transcription of the RNA genome and active transport of the preintegration complex into the nucleus.

Numerous genetic analyses have revealed that the HIV-1 CA protein plays essential roles early and late in the infectious cycle. The C-terminal third of CA (152–231) appears to function primarily as an assembly domain and is required for Gag oligomerization (Franke et al., 1994a), CA dimerization (Gitti et al., 1996), and viral assembly (Jowett et al., 1992; Dorfman et al., 1993; Von Poblotzki et al., 1993; Reicin et al., 1995). In contrast, mutations and deletions in the N-terminal domain of HIV-1 CA (1–151) typically give rise to virions that assemble and bud but are nevertheless noninfectious (Dorfman et al., 1993; Wang and Barklis, 1993; Franke et al., 1994b; Reicin et al., 1995). The correlation between proper core assembly and viral infectivity suggests that the CA core structure plays an essential role early in viral replication.

HIV-1 replication also requires packaging of the cellular peptidyl prolyl isomerase, cyclophilin A (CypA) (Franke et al., 1994b; Thali et al., 1994). CypA binds directly to the CA domain of Gag (and subsequently to the processed CA protein), with a CA–CypA stoichiometry of approximately 2000:200 per virion. Formation of the CA/CypA complex is competitively inhibited by molecules that bind in the active site of CypA, including the immunosuppressive drug cyclosporine and its nonimmunosuppressive analog SDZ NIM 811 (Luban et al., 1993; Billich et al., 1995). Complex formation can also be inhibited by a series of mutations in the N-terminal domain of CA (Franke et al., 1994b; Braaten et al., 1996b). Reagents and mutations that inhibit the CA–CypA interaction in vitro also block CypA packaging and HIV-1 replication in culture, demonstrating that this interaction is essential for viral infectivity (Franke et al., 1994b; Thali et al., 1994; Traber et al., 1994; Steinkasserer et al., 1995; Braaten et al., 1996b).

In addition to packing CypA, CA also appears to be the functional target of CypA. For example, selected mutations in residues located immediately beyond the CypA binding site confer viral resistance to SDZ NIM 811 (Aberham et al., 1996; Braaten et al., 1996a). Intriguingly, these mutant viruses can still package CypA but no longer require the protein to replicate (Braaten et al., 1996a). In fact, their replication actually appears to be inhibited by CypA, since they require SDZ NIM 811 to replicate in some cell lines (Aberham et al., 1996).

The precise function of CypA in HIV-1 replication is unknown. Indeed, despite extensive study, the normal cellular functions of CypA and other cyclophilins are not yet well understood (Stamnes et al., 1992). CypA is ubiquitiously expressed in the cytoplasm of eukaryotic cells (Koletsky et al., 1986), while other family members exhibit tissue- or organelle-specific expression patterns or can exist as discrete domains of larger proteins.

*These authors contributed equally to this work.