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Structural insights into the catalytic mechanism of cyclophilin A

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Cyclophilins constitute a ubiquitous protein family whose functions include protein folding, transport and signaling. They possess both sequence-specific binding and proline *cis-trans* isomerase activities, as exemplified by the interaction between cyclophilin A (CypA) and the HIV-1 CA protein. Here, we report crystal structures of CypA in complex with HIV-1 CA protein variants that bind preferentially with the substrate proline residue in either the *cis* or the *trans* conformation. *Cis*- and *trans*-Pro substrates are accommodated within the enzyme active site by rearrangement of their N-terminal residues and with minimal distortions in the path of the main chain. CypA Arg55 guanidinium group probably facilitates catalysis by anchoring the substrate proline oxygen and stabilizing sp^3 hybridization of the proline nitrogen in the transition state.

CypA is the prototypical member of the widespread cyclophilin family of enzymes, which catalyze the *cis-trans* isomerization of peptide bonds preceding proline residues[1,2]. This activity accelerates protein folding *in vitro*[3–5] and may underlie some of the many roles of cyclophilins[6], which include signaling, mitochondrial function, chaperone activity, RNA splicing, stress response, gene expression and regulation of kinase activity. The biological activities of CypA include binding the HIV-1 CA protein and facilitating viral replication. Interaction with the N-terminal domain of the HIV-1 CA protein (CA^N) results in incorporation of CypA into viral particles at a CypA:CA ratio of ~1:10 (refs. 7–9). This seems to be required for replication of all main (M) and some outlier (O) HIV-1 strains[10,11], although the basis for the role of CypA in viral replication remains unclear and the CypA isomerase activity is reportedly not required for HIV-1 infectivity[12].

The recent demonstration that HIV-1 CA^N is a substrate for isomerization by CypA *in vitro*[13] makes it an attractive system to determine the structural basis for this activity. This is important because the isomerase activity is essential for at least some biological functions of cyclophilins[14–16] and because the catalytic mechanism remains controversial. Our previously determined structure of the CypA–CA^N_{NL43} complex (at a resolution of 2.4 Å)[17] and associated binding studies[18] revealed that all contacts to CypA are contained within a short exposed loop centered on CA^N Pro90. Essentially identical interactions were observed for CypA complexes with CA-derived peptides[19,20]. Unlike other CypA–peptide complexes[21–24], the CypA–CA^N structure bound in the *trans* conformation. This seemed to result from the ability of Gly89, the residue preceding the isomeric peptide of Pro90, to bind deeply into the CypA active site cleft, whereas larger side chains would be excluded from this arrangement while in the *trans* conformation[17]. Comparison of the CypA–CA^N structure with CypA–peptide complexes further suggested that catalysis proceeds by rotation of groups N-terminal to the isomeric proline. More recently, however, an NMR study of chemical shift and NMR relaxation rate changes in the presence and absence of a model substrate led to the conclusion that catalysis by CypA is achieved by rotation of the substrate's C-terminal residues while the N-terminal residues remain stationary[25].

In an effort to gain a better understanding of the catalytic mechanism, we have determined crystal structures of CypA complexes with a series of CA^N variants. The structures have been refined at high resolu-

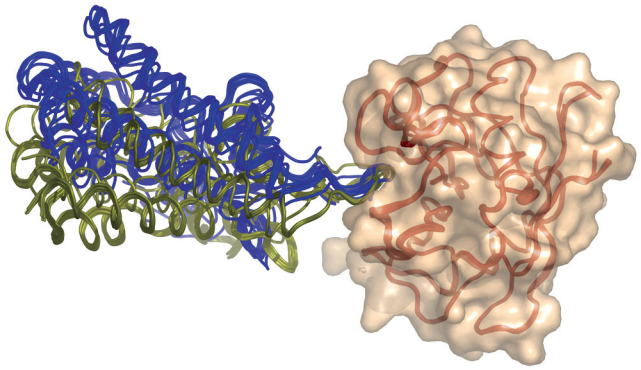

Figure 1 Overall structure of CypA–CA^N complexes. All 16 crystallographically independent structures reported in this paper are shown. Overlaps shown here and in all other figures were obtained by least-squares superposition of main chain atoms of CypA residues 3–165. The hinge angle depends on position in the asymmetric unit; A and A′ CA^N molecules are colored green; B and B′ CA^N, blue; CypA, red with a straw-colored molecular surface. The different hinge angles are accommodated by variation in ϕ and ψ angles over several residues either side of the CA^N Gly89-Pro90 peptide, with the largest change in the wild-type structure for CA^N Met96 ($\Delta\phi = 31°$). **Figures 1**, **2d** and **3** were made with PyMOL (DeLano Scientific; http://www.pymol.org). **Figures 2a–c** and **4** were made with MOLSCRIPT[40] and RASTER3D[41].

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