Cyclophilins have been implicated in such diverse processes as the heat shock response (Sykes et al., 1993), cell surface recognition (Anderson et al., 1993), and various signaling pathways (Bram and Crabtree, 1994; Weisman et al., 1996). In general, cyclophilins are believed to function as chaperones in protein trafficking pathways or as catalysts of protein folding and assembly. The strongest evidence for a chaperone function is provided by the Drosophila cyclophilin, NinaA, which forms a stable complex with its genetically defined substrate, rhodopsin 1 (Rh1). The Rh1/Nina A complex is essential for transporting rhodopsin through the endoplasmic reticulum and into the rhabdomere (Colley et al., 1991; Baker et al., 1994). Cyclophilins may also play more general roles in protein folding since they catalyze *cis*/*trans* isomerization of prolines in model peptides and can accelerate overall protein folding rates as much as 10-fold in vitro (reviewed in Schmid, 1993).

Virions lacking CypA adopt normal morphologies, package wild-type levels of the major viral proteins and RNAs, exhibit normal levels of endogenous reverse transcriptase activity, and appear competent to enter cells (Steinkasserer et al., 1995; Braaten et al., 1996b). Nevertheless, viral replication is blocked at an early step following penetration and cannot be rescued by CypA in the host cell (Thali et al., 1994; Braaten et al., 1996b). This blockage probably occurs prior to the first detectable steps of reverse transcription (Braaten et al., 1996b), although one group has reported that the RNA genome is reverse transcribed but fails to reach the nucleus (Steinkasserer et al., 1995). In either case, CypA appears to act on CA while the virion is uncoating and/or being transported through the cytoplasm, leading to the hypothesis that CypA assists in viral CA core disassembly.

To gain insight into the function of CypA in the HIV-1 life cycle, we have determined the X-ray crystal structure of CypA bound to the N-terminal domain of HIV-1 CA. This is the first example of a cyclophilin complexed with an authentic biological protein partner. The structure reveals the sequence-specific interactions required for HIV-1 packaging of CypA and suggests a mechanism for the nonspecific rotamase activity of CypA. The crystal structure also exhibits CA–CA interactions that could mediate viral CA core assembly and suggests a mechanism by which CypA could assist in disassembling the CA core particle.

Results and Discussion

Structure Determination

Crystals of a complex between CypA (165 residues) and the N-terminal domain of CA (residues 1–151, CA_{151}) were grown, and data were collected to 2.36 Å resolution. There are two CA_{151}/CypA complexes in the asymmetric unit (CA_{151}–1/CypA-1 and CA_{151}-2/CypA-2). The two CypA molecules were positioned by molecular replacement using a 1.64 Å resolution structure of CypA as the search model (Ke et al., 1993). The full structure was determined using the resulting partial model phase information and refined against 6.0–2.36 Å data. The R value is 23.5% (free R value 30.3%) with good stereochemistry. The last six residues of both CA_{151} molecules are disordered and have been omitted from the model. In addition, 26 residues and 32 side chains throughout the two complexes lack defined electron density and have been given an occupancy of zero for crystallographic refinement and for R value calculations. These disordered residues have been included in the model for illustrative purposes and for calculations of quantities such as accessible surface areas.

Conformations of the two CA_{151} molecules and of the two CypA molecules in the asymmetric unit are quite similar. C^{α} atoms overlap with a root mean square deviation (RMSD) of 0.12 Å for CypA and 0.77 Å for CA_{151}. The superposition for CA_{151} improves to 0.43 Å when residues 86–98 are omitted. Neither CypA nor CA_{151} exhibit major changes in conformation upon complex formation. The structures of CypA overlap the starting search model with an RMSD of 0.49 Å and 0.63 Å for C^{α} atoms of CypA-1 and CypA-2, respectively. Overlap of CA_{151} C^{α} atoms with the NMR structure of free CA_{151} (Gitti et al., 1996) is 1.29 Å for CA_{151}-1 and 1.26 Å for CA_{151}-2 when regions that are mobile in solution are neglected.

Structure of CA_{151}

CA_{151} is a flat, tapered molecule with overall dimensions of 18 Å × 35 Å × 45 Å and predominantly helical secondary structure (Figure 1). Helices 1–4 and 7 pack lengthwise, with the perpendicular helices 5 and 6 located at the top of the structure (Figure 1B). An N-terminal β hairpin and a long loop that contains the CypA binding site are also located at the top of the structure.

The N-terminal β hairpin projects away from the main body of the protein, and the charged amino group of Pro-1 forms a buried salt bridge with Asp-51. This structure can presumably only form following proteolytic maturation, since Pro-1 is uncharged in the Gag precursor, and since residues preceding Pro-1 would exhibit unacceptable steric clash in the hairpin conformation (Gitti et al., 1996). It is probable that this conformational change is functionally significant because Pro-1 and Asp-51 are the only residues within the N-terminal domain that are invariant in an alignment of CA proteins from a broad spectrum of retroviruses (McClure, 1991). Based on our crystal structure, we propose that formation of the β hairpin upon proteolytic processing of Gag allows the formation of a new CA–CA interface and may thereby drive formation of the mature CA core structure (see below).

Structure of the CA_{151}/CypA Complex

Cyp A is an eight-stranded antiparallel β barrel, and the active site groove lies on one side of the barrel (Kallen et al., 1991; Ke et al., 1993). In the CA_{151}/CypA complex, the long loop that connects CA_{151} helices 4 and 5 binds across the CypA active site in an extended conformation (Figure 2A). Surprisingly, the ordered CA_{151}/CypA interface is formed exclusively by CA_{151} residues 85–93. The CypA binding loop is one of the most mobile regions of free CA_{151} (Gitti et al., 1996), and loop mobility is also apparent in the CA_{151}/CypA complex. Although the pairs of CypA and CA_{151} molecules in the asymmetric unit superimpose well, the orientations of CypA and CA_{151} in the two complexes differ by approximately 22° (Figure 2B). This difference is best described as a hinge motion