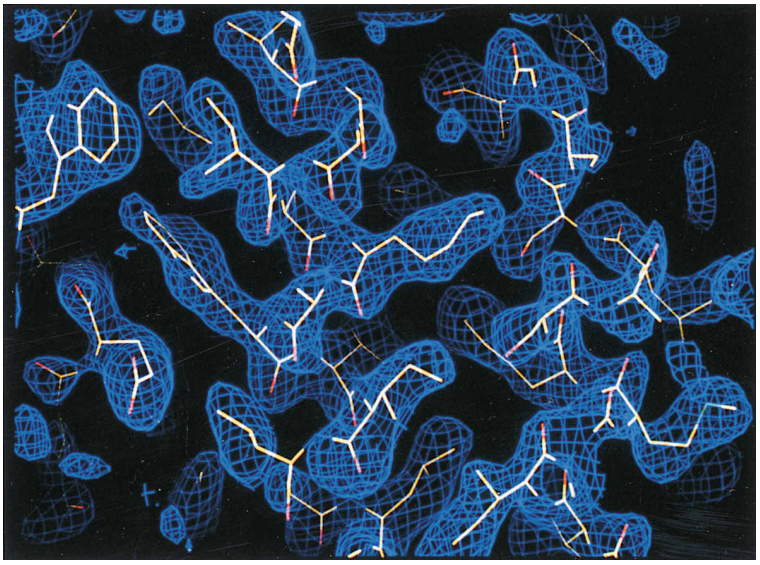

Figure 7. Unbiased Electron Density of CA_{151}

All the protein in this view is CA_{151}, which was not used in the phase calculations. The map, contoured at 0.75 RMSD, was computed at 20–2.36 Å resolution using phases from CypA after rigid body refinement. Weights were computed with SIGMAA, and phases were refined by solvent flattening, histogram shifting, and 2-fold averaging within volumes defined separately for CA_{151} and CypA. Masks for solvent flattening and averaging were defined from the model. This figure was made with O (Jones et al., 1991).

the crystal and would require the formation of only one additional interface. (3) One of the CA–CA interfaces is mediated, in part, by the N-terminal β hairpins, which can only form after proteolysis of the Gag precursor. Thus, this local conformational change may act as a trigger for the global rearrangement of CA into the cone-shaped core during viral maturation (Figure 6A). (4) The position of the CypA binding loop at the top edge of the CA strips suggests a possible mechanism for CypA in destabilizing the viral core (see the following section). (5) CA_{151} is tapered towards its C-terminal end (down in Figure 1B), consistent with curvature toward the inside surface of the core.

Possible Role of CypA in CA Core Disassembly

CypA functions at an early step in the HIV-1 replication cycle, most likely facilitating disassembly of the CA core following entry into the host cell (Braaten et al., 1996b). The observation that the *cis* and *trans* conformations of Pro-90 are in slow exchange in free CA_{151} has suggested that CypA may function to accelerate this isomerization and thereby overcome a kinetic block to cone rearrangement or disassembly (Gitti et al., 1996). The structure does not obviously support this hypothesis, however, since it appears that Pro-90 can be accommodated in the *cis* conformation without any change to the structure of CA_{151} beyond the flexible CypA binding loop. Thus, although the CypA/CA_{151} structure does not rule out a role for the rotamase activity, it is not clear how an increase in the rate of isomerization of Pro-90 would facilitate disassembly of the CA cone.

Our side-to-side model for assembly of the CA strips suggests an alternative mechanism in which the sequence-specific binding of CypA destabilizes CA–CA interactions, thus facilitating core disassembly. The CypA binding loops are located at the top edge of the CA strips, and it appears that binding of CypA sterically inhibits interactions between the strips. In the crystal, where the ratio of CA_{151} to CypA is 1:1, this results in the complete separation of the CA_{151} strips. In the virion, however, where the CA–CypA stoichiometry is 2000:200, CypA may destabilize the core and reduce the cooperativity of disassembly by introducing a series of minor dislocations between the associated strips of CA molecules.

Experimental Procedures

Crystallization and Data Collection

Expression and purification of the recombinant HIV-1 CA_{151} and human CypA were as described (Gitti et al., 1996; Yoo et al., unpublished data). CA_{151}/CypA complex crystals were grown at 21°C in sitting drops. The protein solution was 0.25 mM CypA and 0.25 mM CA_{151} in 10 mM TrisHCl (pH 8.0) and 1 mM 2-mercaptoethanol. The reservoir solution was 1 mL of 1.0 M LiCl, 0.1 M Bicine (pH 7.0), and 22% polyethylene glycol 8000, and the initial 6 μL drop was a 1:1 mix of protein and reservoir solutions. Clusters of small thin plates appeared within a week, and microseeding was used to obtain larger single crystals. Crystals grew to full size (typically 0.5 mm × 0.25 mm × 0.05 mm) over 2 weeks. The space group is $P2_1$, with a = 38.6 Å, b = 113.1 Å, c = 67.0 Å, and β = 100.7°.

Crystals were harvested by transfer to a cryoprotectant solution of 18% glycerol, 1.0 M LiCl, 0.1 M Bicine (pH 7.0), and 26% polyethylene glycol 8000, rapidly suspended in a rayon loop and plunged into liquid nitrogen. X-ray data were collected at a wavelength of 0.978 Å on a MAR Research detector at beam line X12B of the National Synchrotron Light Source (see Table 1). The crystal was maintained at 100°K in a stream of nitrogen gas for data collection. Data were processed with DENZO and SCALEPACK (Otwinowski, 1993).

Structure Determination and Refinement

Crystallographic calculations employed programs from the CCP4 suite except where noted. The two CypA molecules in the asymmetric unit were located by molecular replacement with AMoRe (Navaza,

Table 1. Data Processing Statistics

Resolution (Å)	20.0–2.36	2.40–2.36
# Reflections[a]	21,503	1049
Completeness (%)	97	91
Rsym(I)[b] (%)	11.0	39.9
<I/sig(I)>	10.5	3.5

[a] measured as 318,604 partial and 8039 full observations.

[b] Rsym = $100 * \Sigma_{hkl}\Sigma_i|I_i - \langle I\rangle| / \Sigma\langle I\rangle$

A total of 193° of data were collected. The majority of oscillation ranges were 0.5°, with 300–420 sec exposure time. The mosaicity was 0.783°.