to that observed upon CAP-1 binding, thereby allowing residues of CA^C to occupy the CAP-1 binding pocket. This mechanism would provide a biological explanation for the strained Phe32 backbone and the observed conformational switch, and would assume that CAP-1 functions as a direct competitor for the Phe32 pocket. Additional studies will be required to evaluate these or other potential CA^N-CA^C binding modes.

Potential for future drug development

Although CAP-1 exhibits antiviral activity in cell cultures at non-toxic doses, its affinity for CA^N (0.8 mM) is significantly below the levels needed for therapeutic use. The structure reported here of the CAP-1:CA^N complex provides details that may be useful for developing new assembly inhibitors with improved affinities. The cavity vacated by Phe32 encloses a volume of 264 $Å^3$ and presents a total of 249 $Å^2$ of Connoly molecular surface to its inner walls.[42] The buried portion of CAP-1 occupies only 194 $Å^3$ (73%) of the cavity volume in our joint NMR/X-ray structure, and modifications that improve the fit to the binding site should greatly improve inhibitor binding. The carbonyl oxygen of Ala31 is located within the largely hydrophobic pocket, and modifications that enable hydrogen bonding with this buried oxygen atom should similarly enhance binding. In addition, the backbone oxygen atom of Val59 and the NH groups of Gly61 and His62 are available for hydrogen bonding at the mouth of the cavity, and these groups might also be exploited to enhance binding affinity.

The discovery and development the non-nucleoside reverse transcriptase inhibitors (NNRTIs) followed a similar pathway. The NNRTIs bind to a pocket that forms only in the presence of inhibitors[43–45] and involves reorientation of aromatic sidechains from native buried positions.[44,45] In addition, the initially discovered NNRTIs exhibited relatively poor affinities for RT, but the affinities were substantially improved by structure-based drug design (for example, see Artico *et al.*[46]). These similarities provide grounds for optimism that useful inhibitors that target the CAP-1 binding site can be developed following a similar strategy. An unfortunate problem with the NNRTIs is that their binding site can readily accommodate mutations, and this has led to the development of resistance to this class of inhibitors. Because Phe32 is highly conserved, it is conceivable that the CAP-1 binding site may be less susceptible to drug-induced evolutionary pressure.

In summary, we have determined the structure of the CA^N:CAP-1 complex and identified structural features that may be exploited to enhance binding affinity. Binding involves a major reorientation of Phe32, which appears to be promoted by main-chain strain. We speculate that this strain may be evolutionarily conserved to allow structural changes associated with CA^N-CA^C interactions during capsid assembly, and that CAP-1 binding interferes with these interactions. Efforts to develop new inhibitors with improved efficacy using the CA^N:CAP-1 structure as a guide are underway.

Materials and Methods

Structure determination of CA^N crystallized in the presence of CAP-1

CA^N was prepared using a published procedure,[47] except that a final step was added in which protein was dialyzed into 10 mM Tris (pH 8.0), 50 mM NaCl, 5 mM β-mercaptoethanol and run on a S75 (Pharmacia) sizing column. Crystals were obtained overnight at 21 °C, 13 °C and 4 °C conducted by mixing solutions of CA^N (0.84 mM, with 10 mM Tris (pH 8.0), 50 mM NaCl, 5 mM β-mercaptoethanol) and CAP-1 (Maybridge Chemicals, Cornwall, England, 15.7 mM in DMSO-d_6) ([CA^N] = 0.72 mM; CAP-1:CA^N ratio = 6.1:1). Crystals grew in sitting drops that were prepared using a Hydra 96+1 crystallization robot (Robbins Scientific, Sunnyvale, California). The drop was a mixture of 0.5 μl CA^N:CAP-1 solution and 0.5 μl reservoir (100 mM Tris (pH 8.5), 5% (w/v) PEG 8000, 20% PEG 300, and 10% (v/v) glycerol). Data were collected from several crystals grown at each of these temperatures; in all cases the diffraction and map quality were similar, although the best data (reported here) was from a crystal grown at 13 °C. Crystals were mounted in a nylon loop and flash-cooled in liquid nitrogen without use of an additional cryo-protectant. X-ray diffraction data were processed with MOSFLM.[48] The single CA^N molecule in the asymmetric unit was located by molecular replacement using MOLREP.[49] Refinement was performed using REFMAC5[50] and map fitting was with XTALVIEW.[51] Figures were made with PyMol§.

Crystallization and structure determination of CA^N A92E

CA^N(A92E) was prepared and purified as described.[12] The sequence (Gag residues 133–278; CA residues 1–146) corresponds to wild-type HIV-1_{NL4-3} CA^N, except that Ala92 was replaced by Glu. The purified protein was dialyzed against 10 mM Tris (pH 8.0), 50 mM NaCl, 2 mM β-mercaptoethanol, run on a S75 gel filtration column (Pharmacia), and concentrated to 0.9 mM. Crystals grew after several weeks at 21 °C in sitting drops with a reservoir solution of 24% PEG 4500, 0.60 M $MgCl_2$, and 100 mM Tris–HCl (pH 8.5), and a drop of two parts protein solution to one part reservoir solution.[52] Crystals were briefly transferred to a cryo-protectant consisting of well solution supplemented with 10% glycerol, then suspended in a nylon loop and flash-cooled in liquid nitrogen. HKL and SCALEPACK[53] were used for data processing. The structure was determined *via* molecular replacement with PHASER[54] using a previously solved HIV-1 CA^N as the search model. Refinement used REFMAC5[50] in the CCP4 suite of programs.[55] Model building was done with Coot.[56]

Inspection of the diffraction pattern and indexing trials revealed that half of the reflections are systematically weak, indicating the presence of translational pseudo-

§ http://pymol.sourceforge.net/