The reservoir solution was 15–25% (w/v) PEG 8K, 1 M LiCl and 100 mM Bicine, pH 7.0–9.0. Protein and reservoir solutions were mixed at a 1:1 ratio and microseeded. Crystals typically grew in radial clusters as elongated plates or prisms, reaching a maximal size (~$0.1 \times 0.5 \times 0.5$ mm^3 or $0.1 \times 0.1 \times 0.5$ mm^3) within 2 weeks. Single crystals were harvested by transferring briefly to a cryoprotectant solution, suspended in a rayon loop and cooled by plunging into liquid nitrogen. The cryoprotectant consisted of 11–26% (w/v) PEG 8K, 1 M LiCl, 100 mM Bicine, pH 7.0–8.0, and 10% (v/v) butanediol.

Data collection and processing. All diffraction data were collected at 100 K. Data for WT, AAG, AMG, AAA, AMA and O-loop structures were processed with DENZO and SCALEPACK[32]. Data for AAG were processed with MOSFLM and SCALA[33]. Data for AAA and AMA did not merge well in space group $P2_1$, giving R_{sym} values of 12.3% and 10.6%, respectively, in the low-resolution bins (16.0% and 13.2% overall). Reprocessing of these data in space group $P1$ gave much better statistics. The corresponding reduction to ~2.1-fold data redundancy does not account for the drastically lowered R_{sym} values in $P1$, because scaling in $P2_1$ of truncated data sets that have ~2-fold redundancy results in R_{sym} values in the low-resolution bins that remain at 10.9% and 8.8%, respectively (15.0% and 11.1% overall). Processing of data for WT, AAG, AMG and O-loop structures in space group $P1$ did not result in a significant reduction in R_{sym} values. Crystallographic statistics are given in **Table 3**.

Crystallographic refinement. Refinements were initiated with rigid-body minimization of the WT CypA–CA^N(1–151) model (PDB accession code 1AK4)[17], from which water molecules were deleted. CA^N residues 85–95 were given zero occupancy, and CA^N and CypA molecules were treated as independent units. Crystallographic computing made extensive use of the CCP4 suite[34]. Initial refinement was done with X-PLOR[35]. For all structures, inspection of $F_o - F_c$ difference maps revealed clearly interpretable electron density for the omitted loop, which was built into the electron density using O[36], and water added. For AMG, AAA and AMA, solvent structure was built using ARP[37] and REFMAC[38]. Final refinement of all structures was completed using REFMAC5 (ref. 39) with a maximum-likelihood target function and a Babinet-type bulk solvent correction. Riding hydrogen atoms were placed during refinement, but not written to the output coordinate files. During final refinement rounds of the AMA structure, a minor CA^N Ala89-Pro90 *cis*-proline conformation was apparent for the two loops that had been initially modeled as *trans*. These were modeled as alternate conformations for CA^N residues 88–91 and refined using a version of REFMAC5, modified for this purpose. Residues 89 and 90 of the minor *cis* conformations overlap closely with the fully occupied *cis* structures of the AMG and O-loop structures although, presumably because of the low occupancy, the minor conformations were not very stable in refinement. The occupancies were adjusted manually so that the temperature factors of residues 88 and 91 refined to similar values for the two conformations. The two loops showing this disorder in AMA were estimated to be ~80% *trans* and 20% *cis*. Difference density for AAA-A was also suggestive of a similar minor conformation, although in that case the occupancy was too low for reliable model building.

We further investigated the crystal structure of a CypA–tetrapeptide complex that has been used as the starting point for a molecular dynamics simulation[31]. This structure was reported to show twists of 21° and 14° from *cis* planarity for the two copies in the asymmetric unit[23], although these values are expected to be overestimates because this structure, which was published some years ago, was refined at that time in the presence of weak restraints toward the *trans* conformation[23]. We subsequently refined this structure with conventional weights using the same procedure as for the CypA–CA^N complexes, and found that both X-Pro peptides differ by only 13° from perfect *cis* planarity after one round (50 cycles) of refinement. Unfortunately, these data are not of sufficient resolution to permit a reasonable unrestrained refinement.

Coordinates. The refined atomic coordinates and processed structure factor amplitudes have been deposited in the Protein Data Bank with the following accession codes: WT, 1M9C; O-loop, 1M9D; AAG, 1M9E; AMG, 1M9F; AAA, 1M9Y; AMA, 1M9X.

ACKNOWLEDGMENTS

We thank D.R. Davis, S.L. Alam, T.E. Cheatham III and members of the Hill and Sundquist labs for comments on this manuscript; H. Ke for providing processed structure factor amplitudes for the CypA–tetrapeptide structure; H.L. Schubert for help with refinement; and G.N. Murshudov for modifying REFMAC5 to allow the refinement of peptide structures containing prolines with partial *cis* and partial *trans* conformations. Operations of the Advanced Light Source, National Synchrotron Light Source and Stanford Synchrotron Radiation Laboratory are supported by the U.S. Department of Energy, Office of Basic Energy Sciences, and by the National Institutes of Health. This work was supported by NIH grants to W.I.S. and C.P.H.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 15 October 2002; accepted 28 March 2003
Published online 5 May 2003; corrected 12 May 2003 (details online);
doi:10.1038/nsb927

1. Fischer, G., Whittmann-Liebold, B., Lang, K., Kiefhaber, T. & Schmid, F.X. Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* **337**, 476–478 (1989).
2. Takahashi, N., Hayano, T. & Suzuki, M. Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A–binding protein cyclophilin. *Nature* **337**, 473–475 (1989).
3. Schmid, F.X., Mayr, L.M., Mücke, M. & Schönbrunner, E.R. Prolyl isomerases: role in protein folding. *Adv. Protein Chem.* **44**, 25–66 (1993).
4. Kern, G., Kern, D., Schmid, F.X. & Fischer, G. A kinetic analysis of the folding of human carbonic anhydrase II and its catalysis by cyclophilin. *J. Biol. Chem.* **270**, 740–745 (1995).
5. Kern, D., Kern, G., Scherer, G., Fischer, G. & Drakenberg, T. Kinetic analysis of cyclophilin-catalyzed prolyl *cis/trans* isomerization by dynamic NMR spectroscopy. *Biochemistry* **34**, 13594–13602 (1995).
6. Gothel, S.F. & Marahiel, M.A. Peptidyl-prolyl *cis-trans* isomerases, a superfamily of ubiquitous folding catalysts. *Cell. Mol. Life Sci.* **55**, 423–436 (1999).
7. Luban, J., Bossolt, K.L., Franke, E.K., Kalpana, G.V. & Goff, S.P. Human immunodeficiency virus type 1 gag protein binds to cyclophilins A and B. *Cell* **73**, 1067–1078 (1993).
8. Franke, E.K., Yuan, H.E.H. & Luban, J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* **372**, 359–362 (1994).
9. Thali, M. *et al.* Functional association of cyclophilin A with HIV-1 virions. *Nature* **372**, 363–365 (1994).
10. Braaten, D., Franke, E.K. & Luban, J. Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus $SIV_{CPZ}GAB$ but not group O HIV-1 or other primate immunodeficiency viruses. *J. Virol.* **70**, 4220–4227 (1996).
11. Wiegers, K. & Kräusslich, H.-G. Differential dependence of the infectivity of HIV-1 group O isolates on the cellular protein cyclophilin A. *Virology* **294**, 289–295 (2002).
12. Saphire, A.C., Bobardt, M.D. & Gallay, P.A. *Trans*-complementation rescue of cyclophilin A-deficient viruses reveals that the requirement for cyclophilin A in human immunodeficiency virus type 1 replication is independent of its isomerase activity. *J. Virol.* **76**, 2255–2262 (2002).
13. Bosco, D.A., Eisenmesser, E.Z., Pochapsky, S., Sundquist, W.I. & Kern, D. Catalysis of *cis/trans* isomerization in native HIV-1 capsid by human cyclophilin A. *Proc. Natl. Acad. Sci. USA* **99**, 5247–5252 (2002).
14. Brazin, K.N., Mallis, R.J., Fulton, D.B. & Andreotti, A.H. Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. *Proc. Natl. Acad. Sci. USA* **99**, 1899–1904 (2002).
15. Arévalo-Rodríguez, M., Cardenas, M.E., Wu, X., Hanes, S.D. & Heitman, J. Cyclophilin A and Ess1 interact with and regulate silencing by the Sin3–Rpd3 histone deacetylase. *EMBO J.* **19**, 3739–3749 (2000).
16. Schmid, F.X. Prolyl isomerases. *Adv. Prot. Chem.* **59**, 243–282 (2002).
17. Gamble, T.R. *et al.* Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* **87**, 1285–1294 (1996).
18. Yoo, S. *et al.* Molecular recognition in the HIV-1 capsid/cyclophilin A complex. *J. Mol. Biol.* **269**, 780–795 (1997).
19. Zhao, Y., Chen, Y., Schutkowski, M., Fischer, G. & Ke, H. Cyclophilin A complexed with a fragment of HIV-1 gag protein: insights into HIV-1 infectious activity. *Structure* **5**, 139–146 (1997).
20. Vajdos, F., Yoo, S.-H., Houseweart, M., Sundquist, W.I. & Hill, C.P. Crystal structure of cyclophilin A complexed with a binding site peptide from the HIV-1 capsid protein. *Protein Sci.* **6**, 2297–2307 (1997).
21. Kallen, J. *et al.* Structure of human cyclophilin and its binding site for cyclosporin A determined by X-ray crystallography and NMR spectroscopy. *Nature* **353**, 276–279 (1991).
22. Ke, H., Mayrose, D. & Cao, W. Crystal structure of cyclophilin A complexed with substrate Ala-Pro suggests a solvent-assisted mechanism of *cis-trans* isomerization. *Proc. Natl. Acad. Sci. USA* **90**, 3324–3328 (1993).
23. Zhao, Y. & Ke, H. Crystal structure implies that cyclophilin predominantly catalyzes the *trans* to *cis* isomerization. *Biochemistry* **35**, 7356–7361 (1996).
24. Zhao, Y. & Ke, H. Mechanistic implications of crystal structures of the cyclophilin–dipeptide complexes. *Biochemistry* **35**, 7362–7368 (1996).
25. Eisenmesser, E.Z., Bosco, D.A., Akke, M. & Kern, D. Enzyme dynamics during catalysis. *Science* **295**, 1520–1523 (2002).
26. Gitti, R.K. *et al.* Structure of the amino-terminal core domain of the HIV-1 capsid protein. *Science* **273**, 231–235 (1996).