nature structural & molecular biology

Key interactions in HIV-1 maturation identified by hydrogen-deuterium exchange

Jason Lanman[1,5], TuKiet T Lam[2,3], Mark R Emmett[2,3], Alan G Marshall[2,3], Michael Sakalian[4] & Peter E Prevelige Jr[1]

To characterize the intersubunit interactions underlying assembly and maturation in HIV-1, we determined the amide hydrogen exchange protection pattern of capsid protein in the immature virion and the mature virion using mass spectrometry. Alterations in protection upon maturation provide evidence for the maturation-induced formation of an interaction between the N- and C-terminal domains in half of the capsid molecules, indicating that only half of the capsid protein is assembled into the conical core.

Human immunodeficiency virus type 1 (HIV-1) capsids exist in both immature and mature forms. The immature capsid is spherical in morphology, with the Gag polyprotein arranged radially[1,2]. Upon cleavage-induced maturation, the capsid (CA) protein collapses to form a conical core, which encases the complex of nucleocapsid protein (NC) and the dimeric RNA, while the matrix (MA) protein remains associated with the viral envelope[3–5]. Formation of the mature viral core is critical for viral infectivity[6–9].

Despite considerable effort, structural studies of immature and mature virions have lacked sufficient resolution to identify intersubunit interactions. A model of the mature form has been derived from tubes of CA polymerized *in vitro*[10] and refined by mutational[11] and hydrogen-deuterium (H/D) exchange studies[12]. Collectively, these studies provide support for homologous CA N-terminal domain (NTD) and C-terminal domain (CTD) interactions as well as an NTD-CTD interaction in the formation of the core. To determine which of these interfaces is present in the immature virion, we carried out comparative H/D exchange studies of intact immature and mature virus-like particles.

Immature and mature virus-like particles (iVLP and mVLP) lacking envelope protein (Env) were generated, purified and analyzed by SDS-PAGE (see **Supplementary Methods** and **Supplementary Fig. 1** online). To detect conformational heterogeneity within mVLP, the amide H/D exchange pattern of CA (**Fig. 1a**) was analyzed by online liquid chromatography electrospray ionization mass spectrometry (LC-MS) and compared with that of CA in solution. As expected, both forms of CA initially have the same mass (25,601 Da), represented by the peak at $m/z \approx 915.4$. Over time, the mass of CA shifts to a higher value of m/z, reflecting deuterium incorporation. After 30 s of exchange, the peak for free CA in solution is unimodal and has shifted to $m/z = 918.3$, an 84-Da mass increase. In contrast, the peak for CA in mVLP shows a bimodal distribution composed of nearly equal numbers of fast (CA_{fast}) and slow (CA_{slow}) exchanging molecules.

The exchange profile for CA_{fast} is similar to that of free CA in solution (**Fig. 1a**) suggesting that CA_{fast} is not substantially protected relative to free CA. At early exchange periods (≤5 min) we observed a small but reproducible level of protection in CA_{fast} relative to free CA (**Fig. 1b**). It has been suggested that mVLP CA should be dimeric[13], whereas the free CA in solution is predominately monomeric. The degree of protection (~17 Da) corresponds well with the observation that 21 residues become buried upon dimerization[14].

CA_{slow} shows an additional 34-Da protection compared with CA_{fast}; this is consistent with CA_{slow} forming stable NTD interactions similar to those observed in *in vitro*–assembled CA (~40 residues in

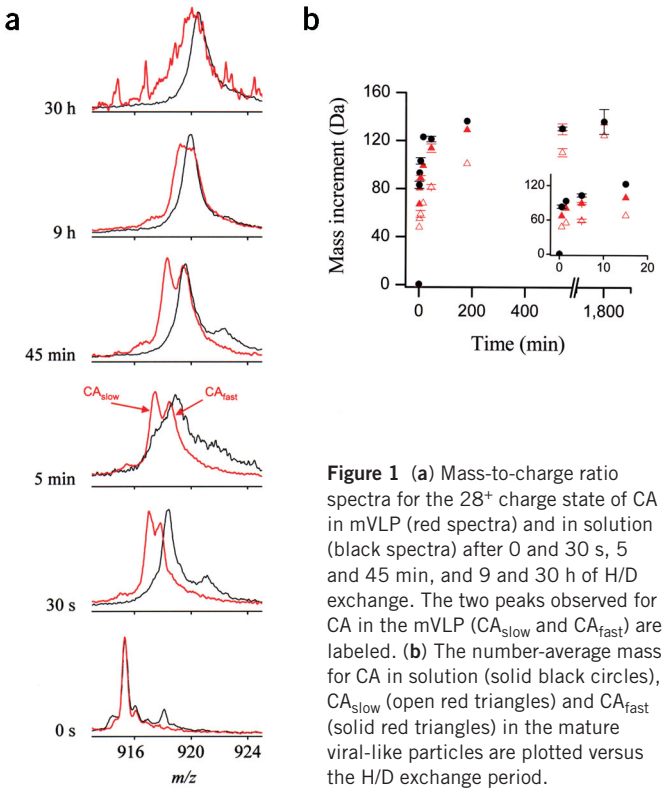

Figure 1 (**a**) Mass-to-charge ratio spectra for the 28^+ charge state of CA in mVLP (red spectra) and in solution (black spectra) after 0 and 30 s, 5 and 45 min, and 9 and 30 h of H/D exchange. The two peaks observed for CA in the mVLP (CA_{slow} and CA_{fast}) are labeled. (**b**) The number-average mass for CA in solution (solid black circles), CA_{slow} (open red triangles) and CA_{fast} (solid red triangles) in the mature viral-like particles are plotted versus the H/D exchange period.

[1]Department of Microbiology, University of Alabama at Birmingham, Birmingham Alabama 35294, USA. [2]Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306, USA. [3]National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida 32310, USA. [4]Department of Microbiology and Immunology, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma 73190, USA. [5]Present address: Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, USA. Correspondence should be addressed to P.E.P. (prevelige@uab.edu).

Published online 20 June 2004; doi:10.1038/nsmb790

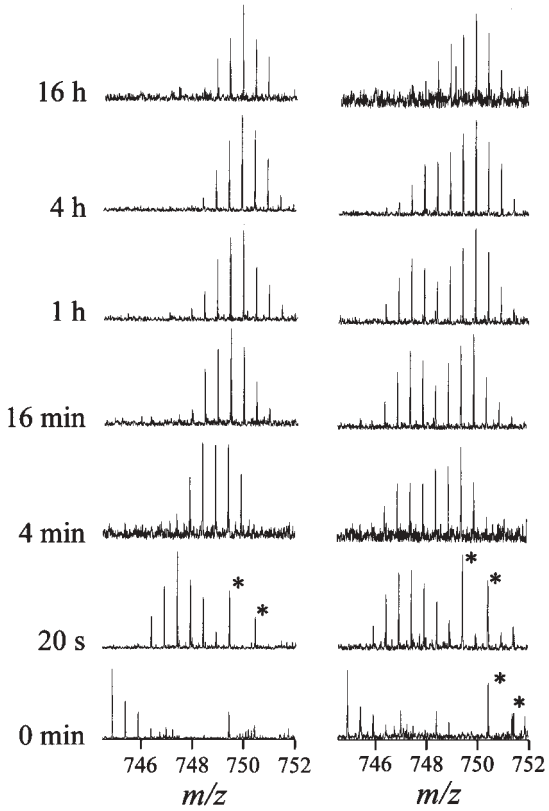

Figure 2 ESI FT-ICR mass spectra for the peptide spanning CA residues 55–68 in iVLP (left) and mVLP (right) after H/D exchange. The peaks labeled with asterisks are not a part of the isotope distribution for this peptide.

the NTD)[12]. This analysis suggests that CA_{slow} molecules form both NTD and CTD interactions, presumably within the conical core. The CA_{fast} and CA_{slow} peaks are comparable in area at all exchange periods, indicating that about half of the CA in mVLP is in the slow-exchanging form and the other half is in the fast-exchanging form, and that the two forms do not interconvert. Using electron microscopy, Briggs *et al.*[15] have independently obtained evidence that only a subpopulation of the total CA molecules in the virion contribute to the mature viral core.

To locate the regions in CA responsible for the bimodal distribution, exchanged iVLP and mVLP were digested with pepsin and the extent of deuterium incorporation into each peptide was analyzed by high-resolution mass spectrometry[12,16]. The peptide spanning CA residues 55–68 ($m/z = 744.84$) corresponding to the NTD-CTD interface identified *in vitro*[12] shows a bimodal distribution in the mature virion but not in the immature virion (**Fig. 2**). The H/D exchange profiles of this segment in iVLP and the faster component of mVLP (**Fig. 2**) are very similar to that seen for unassembled CA[12], indicating that these CA molecules are probably similar in conformation to CA from unassembled subunits. The exchange profile for the slower-exchanging component in mVLP is similar to that previously observed for *in vitro*–assembled CA[12], suggesting that the core-associated CA molecules form the heterotypic NTD-CTD interaction in mVLP. The relative distribution of fast- and slow-exchanging subpopulations agrees well with that observed in intact CA and suggests that this interface is present in the bulk of the core-associated molecules, independent of their location.

CA helices I (residues 16–30) and II (residues 33–47) have been implicated in the formation of the mature virion on the basis of EM reconstructions of *in vitro*–assembled tubes[10]. The degree of protection for residues 1–22 observed in mVLP is similar to that observed in *in vitro* tubes, suggesting that a similar homotypic NTD interaction is present in mVLP (see **Supplementary Fig. 2** online).

A peptide corresponding to residues 23–40 (see **Supplementary Fig. 2** online) was observed in all three forms (iVLP, mVLP and *in vitro*). *In vitro*, the formation of homotypic NTD interactions upon assembly results in a substantial increase in the protection of this peptide[12]. At 100 min of exchange (the time of maximum difference between unassembled and assembled CA), this peptide shows protection in both iVLP and mVLP comparable to that observed in *in vitro*–assembled CA. This result is inconsistent with the peptide being solvent-exposed and suggests that similar NTD interactions are formed in both iVLP and mVLP. Similarly, a peptide corresponding to residues 25–68 is well protected in both iVLP and mVLP, and the peptide corresponding to residues 112–128 shows similar exchange in all assembly states (see **Supplementary Fig. 2** online). No evidence of bimodal exchange was observed for any of these peptides.

Taken together, these results suggest that helices I and II are involved in intersubunit interactions in both the immature and mature virion. A defining characteristic of core formation seems to be the formation of the heterotypic NTD-CTD interaction. In support of this model, mutations in this interface region lead to noninfectious virions, and a small molecule compound that binds near the interface interferes with core formation but not assembly[17].

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

This work was supported by grants from the US National Institutes of Health (AI44626) and the US National Science Foundation (CHE-99-09502), Florida State University and the US National High Magnetic Field Laboratory.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 21 January; accepted 4 May 2004
Published online at http://www.nature.com/nsmb/

1. Wilk, T. *et al. J. Virol.* **75**, 759–771 (2001).
2. Fuller, S.D., Wilk, T., Gowen, B.E., Krausslich, H.G. & Vogt, V.M. *Curr. Biol.* **7**, 729–738 (1997).
3. Hunter, E. *Semin. Virol.* **5**, 71–83 (1994).
4. Freed, E.O. *Virology* **251**, 1–15 (1998).
5. Turner, B.G. & Summers, M.F. *J. Mol. Biol.* **285**, 1–32 (1999).
6. Dorfman, T., Bukovsky, A., Ohagen, A., Hoglund, S. & Gottlinger, H.G. *J. Virol.* **68**, 8180–8187 (1994).
7. Reicin, A.S., Ohagen, A., Yin, L., Hoglund, S. & Goff, S.P. *J. Virol.* **70**, 8645–8652 (1996).
8. von Schwedler, U.K. *et al. EMBO J.* **17**, 1555–1568 (1998).
9. Fitzon, T. *et al. Virology* **268**, 294–307 (2000).
10. Li, S., Hill, C.P., Sundquist, W.I. & Finch, J.T. *Nature* **407**, 409–413 (2000).
11. Ganser-Pornillos, B.K., von Schwedler, U.K., Stray, K.M., Aiken, C. & Sundquist, W.I. *J. Virol.* **78**, 2545–2552 (2004).
12. Lanman, J. *et al. J. Mol. Biol.* **325**, 759–772 (2003).
13. Briggs, J.A., Wilk, T., Welker, R., Krausslich, H.G. & Fuller, S.D. *EMBO J.* **22**, 1707–1715 (2003).
14. Worthylake, D.K., Wang, H., Yoo, S., Sundquist, W.I. & Hill, C.P. *Acta Crystallogr. D* **55**, 85–92 (1999).
15. Briggs, J.A.G. *et al. Nat. Struct. Mol. Biol.* **11**, 672–675 (2004).
16. Lam, T.T. *et al. J. Chromatogr. A* **982**, 85–95 (2002).
17. Tang, C. *et al. J. Mol. Biol.* **327**, 1013–1020 (2003).