Hydrogen Bonding at a Conserved Threonine in Lentivirus Capsid Is Required for Virus Replication

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The N terminus of the capsid protein (CA) undergoes a considerable conformational change when the human immunodeficiency virus (HIV) protease cleaves it free from the Pr55Gag polyprotein. This rearrangement is thought to facilitate the establishment of specific CA-CA interactions that are required for the formation of the mature viral core. Substitution of amino acids that are critical for this refolding of the N terminus is generally detrimental to virus replication and mature virion core morphology. Here, we identify a conserved threonine in simian immunodeficiency virus (SIV) CA, T(47)CA, that is requisite for viral replication. Replacement of T(47)CA in the infectious viral clone SIVmac239 with amino acids with different hydrogen-bonding capabilities and analysis of the effects of these substitutions at key steps in the viral life cycle demonstrate that hydrogen bonding at this position is important for virus infectivity and virion release. In the HIV-based homology model of the mature SIV CA N terminus presented in this study, T(47)CA forms several hydrogen bonds with a proximal aspartate, D(50)CA. This model, coupled with strong phenotypic similarities between viral substitution mutants of each of these two residues in all of the virological assays described herein, indicates that hydrogen bonding between T(47)CA and D(50)CA is likely required for viral replication. As hydrogen bonding between these two residues is present in HIV CA as well, this interaction presents a potential target for antiviral drug design.

The retroviral Gag protein is necessary and sufficient to direct assembly and budding of viral particles (9, 19, 28). In human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), the gag gene is expressed as a polyprotein, Pr55Gag, which contains (from N to C terminus) the matrix (MA), capsid (CA), p2, nucleocapsid, p1, and p6 proteins (25, 26, 33, 36). Pr55Gag is targeted to and interacts with the plasma membrane via a myristic acid moiety at the N terminus of the MA domain, in conjunction with several downstream basic residues (5, 38, 49, 60), and it is there that the virion assembles and buds from the cell (for a review, see reference 27). At an undetermined step in the assembly and budding process, the viral protease cleaves Pr55Gag into its constituent proteins. This cleavage results in a significant morphological change in the virion termed maturation (reviewed in reference 54). In the immature virion, a shell of Pr55Gag lines the inner leaflet of the lipid bilayer comprising the viral envelope. Upon maturation, MA remains associated primarily with the viral envelope, while CA collapses into a conical core surrounding nucleocapsid (20, 50, 55). Formation of the mature viral core is also present in the other complete, innate mature CA structures reported thus far, those of human T-cell leukemia virus type 1 (8, 31) and Rous sarcoma virus (6, 32) CA. The conservation of the salt bridge and β-hairpin suggests that these structural features are important for CA function. A single role has been proposed for the N-terminal β-hairpin in mature CA: interactions between the β-hairpins of CA monomers may facilitate dimerization and core assembly (15, 20, 23, 55). Amino acid substitutions in HIV CA that disrupt the salt bridge, thereby destabilizing the β-hairpin, cause a variety of viral defects, including abnormal mature core morphology and loss of infectivity (13, 51, 55). Because the conformational change in the CA N terminus is essential for proper CA function in the virus life cycle, the interactions that facilitate the formation of the β-hairpin and the salt bridge in mature CA are important potential targets for antiviral drug design (50; reviewed in reference 29).

In this study, we identified a conserved threonine [T(47)CA] required for replication of SIVmac239. The results of virolog-
ical assays using a panel of polar and nonpolar substitutions of T(47)CA in SIV CA demonstrate that hydrogen bonding at this position is critical for the proper function of CA in viral infectivity and virion release. In addition, strong phenotypic similarities were observed between alanine substitution mutants of T(47)CA and a proximal aspartate, D(50)CA. Using the coordinates of the HIV CA N-terminal domain (15), we constructed a homology model of SIV CA. In this model, T(47)CA and D(50)CA are directly hydrogen bonded to each other. This observation, coupled with the phenotypic similarities between T(47)CA and D(50)CA mutants, strongly suggests that hydrogen bonding between T(47)CA and D(50)CA is essential for viral replication. As hydrogen bonding between these two residues is present in HIV CA as well, this interaction may provide a novel target for inhibitors of HIV replication.

MATERIALS AND METHODS

Mutagenesis and cloning. SIVmac239 gag mutants were generated by using the Stratagene Quick Change Site-Directed Mutagenesis kit (Stratagene, Cedar Creek, Tex.). Mutagenesis primers (Keystone Labs, Camarillo, Calif.) were designed according to the recommendations of the QuickChange manufacturer. The codon alterations resulting in amino acid substitutions in MA or CA were GGC→GCA for G(2)ACA, CAC→GAC for P(1)A CA, GCC→ACC for (50)ACA, ACC→GCC for T(47)ACA, ACC→TGC for T(47)CA, ACC→TCC for T(47)CA, and ACC→TTC for T(47)CA. To generate full-length, mutant SIVmac239 in pBS, mutant p239SpSp5' and wild-type p239SpES were both digested with SplI (Invitrogen Corporation, Carlsbad, Calif.) and ligated. All clones were verified by sequencing the complete coding region of the viral genome.

Cell culture and reagents. CEM × 174 cells, a human T-cell/B-cell hybrid line (48) (a generous gift from James Hoxie [University of Pennsylvania]), were cultured as described previously (45). African green monkey kidney COS-1 cells (American Type Culture Collection, Manassas, Va.) were cultured like human embryonic kidney 293T cells (American Type Culture Collection) as described previously (44), except with gentamicin (0.5 mg/ml) instead of penicillin-streptomycin.

Viral growth and infectivity. CEM × 174 cells (5 × 10⁶) were transfected with 12 µg of viral DNA by electroporation at 200 V and 960 µF using a Gene Pulser (Bio-Rad, Hercules, Calif.). Reverse transcriptase (RT) activity in cell-free supernatants from transfected cultures was analyzed at various times posttransfection by using a standard RT assay (7). The infectivity (LuSIV assay; see below) and virion release. In addition, strong phenotypic similarities were observed between alanine substitution mutants of T(47)CA and a proximal aspartate, D(50)CA. Using the coordinates of the HIV CA N-terminal domain (15), we constructed a homology model of SIV CA. In this model, T(47)CA and D(50)CA are directly hydrogen bonded to each other. This observation, coupled with the phenotypic similarities between T(47)CA and D(50)CA mutants, strongly suggests that hydrogen bonding between T(47)CA and D(50)CA is essential for viral replication. As hydrogen bonding between these two residues is present in HIV CA as well, this interaction may provide a novel target for inhibitors of HIV replication.

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RESULTS

Identification of a conserved threonine required for viral replication. In a previous study of the role of capsid phosphorylation in viral budding, we characterized a panel of CA substitution mutants in the infectious viral clone SIVmac239 (J. W. Roos, S. M. Rue, J. E. Clements, and S. A. Barber, submitted for publication). Phosphoamino acid analysis indicated that serine was the predominant phosphoamino acid, and a panel of mutants was created, replacing serines in consensus casein kinase II (CK2) and protein kinase C sites with alanine (Roos et al., submitted). Because threonine is also a potential site of phosphorylation by phosphoamino acid analysis (although to a lesser degree than serine [Roos et al., submitted]), and to ensure that all known potential consensus sites were included in the mutagenesis study, we substituted alanine for each of the two threonines in consensus CK2 sites, in positions 47 [T(47)CA] and 208 [T(208)CA] of CA. Equivalent amounts of proviral DNA from each of these mutants and wild-type SIVmac239 were transfected into CEM × 174 cells, and virus-containing cell supernatants were subjected to an RT assay at various times posttransfection (Fig. 1A). The T(47)CA substitution severely compromised viral replication, while the T(208)CA substitution only modestly delayed replication.

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We next examined the infectivity of virions produced from 293T cells transfected with wild-type and mutant viral DNAs by the LuSIV assay (Fig. 1B). The LuSIV assay, developed in our laboratory (45), is based on a CEM/H11003 cell line stably transfected with a plasmid encoding luciferase under the control of the SIV long terminal repeat. Upon virus infection of this cell line, the viral Tat protein transactivates the long terminal repeat, inducing luciferase expression. Subsequent quantitation of luciferase activity provides a very sensitive measure of virus infectivity. While both substitution mutants were less infectious than wild-type virus in this assay, the infectivity of T(47)ACA virus was approximately fourfold lower than that of T(208)ACA virus.

To investigate whether either of the threonine substitutions affected virion morphology, we transfected 293T cells with wild-type or mutant infectious viral DNA and subjected them to transmission EM analysis. The T(208)ACA particles appeared to be morphologically wild type by EM (data not shown). In contrast, a predominance of the mature particles in photomicrographs of the T(47)ACA sample had acentric cores (core is collapsed and juxtaposed to the viral envelope [see below]).

Alignment of lentiviral Pr55\textsuperscript{gag} protein sequences indicated that T(47)\textsubscript{CA} is well conserved among lentiviruses (Fig. 2), underscoring the importance of this residue and suggesting that it may be similarly required for the replication of other members of this family of retroviruses. Interestingly, in all three virological analyses of the threonine substitution mutants, the T(47)\textsubscript{CA} mutant bore a strong resemblance to previously characterized mutants with substitutions of the two residues that participate in the salt bridge in HIV CA, P(1)LCA and D(51)A\textsubscript{CA}. Like T(47)\textsubscript{CA} SIV, P(1)LCA\textsubscript{CA} and D(51)A\textsubscript{CA} HIV do not replicate well, are noninfectious, and have largely acentric mature cores (13, 51, 55). The similarities between these mutants, in conjunction with the fact that T(47)\textsubscript{CA} is highly conserved (suggesting its functional importance), prompted us to analyze this CA threonine residue in additional experiments.

Because the HIV P(1)\textsubscript{CA} and D(51)\textsubscript{CA} residues are both critical to the structure of the mature CA N terminus (15, 20, 37), we hypothesized that SIV T(47)\textsubscript{CA} may have an important structural role as well. As the structure of SIV CA has not been reported, we investigated the crystal structure of HIV CA (15) to visualize the HIV homologue of SIV T(47)\textsubscript{CA} HIV T(48)\textsubscript{CA}. The most striking feature of this HIV CA structure was that T(48)\textsubscript{CA} participates in four hydrogen bonds with D(51)\textsubscript{CA}: between the T(48)\textsubscript{CA} N and the D(51)\textsubscript{CA} O\textsubscript{H9254}, between the D(51)\textsubscript{CA} N and the T(48)\textsubscript{CA} O\textsubscript{H9253}, between the D(51)\textsubscript{CA} N and the T(48)\textsubscript{CA} O, and between the T(48)\textsubscript{CA} O\textsubscript{Y} and the D(51)\textsubscript{CA} N and the T(48)\textsubscript{CA} O, and between the T(48)\textsubscript{CA} O\textsubscript{Y} and the D(51)\textsubscript{CA} O. By extension, we considered the possibility that hydrogen bonding at T(47)\textsubscript{CA} is structurally important in SIV CA and...
therefore potentially required for proper CA function in the viral life cycle. We hypothesized that SIV T(47)CA hydrogen bonds with D(50)CA, in a manner similar to that observed with HIV.

To test the idea that hydrogen bonding at T(47)CA is required for viral replication, we constructed a panel of polar and nonpolar substitutions of this residue in the full-length viral clone SIVmac239. Two polar amino acids were substituted for T(47)CA in SIVmac239: cysteine [T(47)CCA] and serine [T(47)SCA]. Although both polar residues may form the required hydrogen bonds, these bonds will differ from those potentially formed by T(47)CA in wild-type SIV CA. Another nonpolar substitution [in addition to T(47)ACA] was also made, replacing T(47)CA with valine [T(47)VCA]. Of note, the valine side chain is very similar to that of threonine from a space-filling perspective.

Both T(47)ACA and T(47)VCA would abolish hydrogen bonding of the side chain at position 47 in CA.

Because the HIV CA structure indicates that HIV D(51)CA and T(48)CA are hydrogen bonded to each other, we also substituted alanine for the SIV homologue of HIV D(51)CA [SIV D(50)CA]. We used D(50)ACA as a reference for comparison with the T(47)CA substitution mutants in assays that examine critical steps of viral replication. If T(47)CA and D(50)CA truly are hydrogen bonded to each other in SIV CA, we would expect that T(47)CA and D(50)CA substitution mutants might have similar functional or morphological defects. By analogy to HIV, SIV D(50)CA may participate in a salt bridge with P(1)CA. Since no previous study has investigated whether the sequence homologues of either of the HIV salt bridge partners are critical for SIV replication, for completeness, we substituted alanine for SIV P(1)CA in SIVmac239 [P(1)ACA].

Finally, we changed the glycine at position 2 of the matrix domain to alanine in SIVmac239 [G(2)AMA]. This substitution prevents myristoylation of Pr55Gag and abolishes viral budding (5, 22, 38), providing a negative control for viral replication. To ensure that no other mutations had spontaneously occurred in the cloning process, SIVmac239 mutants were verified by sequencing the entire coding region of the viral genome.

**Growth kinetics of SIVmac239 substitution mutants.** To compare the growth kinetics of our panel of viral mutants, CEM×174 cells were transfected with equivalent amounts of infectious wild-type or mutant viral DNA, and RT activity in virus-containing cell supernatants was measured at various times posttransfection (Fig. 3). P(1)ACA virus consistently reached peak RT activity 6 days later than wild-type virus. In contrast, D(50)ACA virus replicated in only one of three independent transfections (Fig. 3), and in this instance it was delayed by 18 days relative to the wild type and never replicated to wild-type levels.

All of the T(47)CA substitution mutants exhibited severely compromised viral replication: T(47)A CA, T(47)C CA, and T(47)SCA mutants consistently reached peak RT activity 8 to 12 days later than the wild type and never approached wild-type replication levels. The T(47)VCA mutant replicated in only one (data not shown) of three independent transfections, was delayed by 20 days relative to the wild type, and never reached wild-type levels. The observation that all of the T(47)CA substitution mutations inhibited viral replication indicates that a threonine at this position is crucial for the proper function of Gag in the viral life cycle.

Nonpolar substitutions of T(47)CA exhibited greatly reduced infectivity. We next investigated the relative infectivities of virions produced from cells transfected with wild-type SIV and each of our viral mutants using two assays, a standard TCID50 assay (Fig. 4A and B) and the LuSIV assay described above (Fig. 4C). The results for each substitution mutant were consistent in both assays. In keeping with the phenotypes of mutants of the HIV salt bridge participant residues, HIV D(51)ACA and HIV P(1)LCA, D(50)ACA SIV was noninfectious and P(1)ACA SIV exhibited greatly reduced infectivity relative to wild-type virus (13, 51, 55) (Fig. 4). All of the substitutions of T(47)CA...
greatly compromised viral infectivity, although mutants with the polar substitutions T(47)S_CA and T(47)C_CA were more infectious than those with nonpolar substitutions [T(47)V_CA and T(47)A_CA]. The infectivity assay results were consistent for virus derived from both 293T cells (Fig. 4A and C) and COS-1 cells (Fig. 4B and data not shown [LuSIV]), demonstrating that these results are independent of cell type. Collectively, these data indicate that substitution of T(47)_CA, D(50)_CA, or P(1)_CA compromises virion infectivity. Furthermore, nonpolar substitutions of T(47)_CA are more detrimental to virus infectivity than polar substitutions at this position.

Virion release and incorporation of viral proteins. Since the attenuation of viral replication observed for some of the substitution mutants was severe, the observed replication defects could have been manifested not only in virus entry into cells (described above) but also in virion release and/or viral protein incorporation. To assess whether any of the CA substitution mutants were defective in the release of nascent virions, 293T cells were transfected with equivalent amounts of wild-type or mutant SIVmac239 infectious viral DNA and metabolically labeled with [35S]methionine-cysteine. Cell and virus lysates (see Materials and Methods) were immunoprecipitated with SIV CA antiserum and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography (Fig. 5A). As 293T cells are nonpermissive to SIV infection, this assay reflects one round of virus production. The band just above CA in this assay is the CA-p2 cleavage intermediate, similar to that described for Rous sarcoma virus (57) and HIV-1 (53). These gels were subjected to densitometric analysis, and the relative virion release efficiencies for the substitution mutants and wild-type SIV were compared by dividing the total amount of Gag in the virus lysate by the sum of the total amount of Gag in the cell and virus lysates (Fig. 5B).

Consistent with previous studies (5, 22, 38), no Gag was detected in G(2)AMA virus lysates, indicating a severe defect in virion release. The overaccumulation of Pr55<sup>core</sup> observed in cells transfected with G(2)AMA SIV infectious viral DNA is consistent with the phenotype of HIV-1 G(2)AMA (22). While P(1)_CA virus released approximately wild-type levels of Gag, the D(50)_CA mutation released only about 30% as much Gag into the cell supernatant as wild-type virus did, indicating a defect in particle production. Mutants with the nonpolar substitutions T(47)_CA and T(47)V_CA released approximately 90 and 60% as much virus into the cell supernatant as wild-type virus, respectively, while mutants with the polar substitutions T(47)C_CA and T(47)S_CA released approximately wild-type levels of Gag. Similar results were obtained for the panel of mutants when cell and virus lysates from transfected 293T or COS-1 cells were analyzed directly by Western blot analysis with SIV CA antiserum or SIV antiserum (data not shown).

Based on the infectivity data (Fig. 4), the Gag that is released by the budding-deficient D(50)_CA, T(47)_CA, and T(47)V_CA mutants does not likely represent infectious virus. The virion release defects observed for D(50)_CA, T(47)_CA, and T(47)V_CA were somewhat unexpected, given that defective cell exit is not typical of substitutions in the CA N terminus (11, 43, 44, 56). However, our group has recently characterized several other substitution mutants in this region of CA that also exhibit release defects (Roos et al., submitted), and a mutant with a substitution for proline 34 of HIV-1 CA is

![Figure 4](http://example.com/fig4.png)
similarly deficient in particle production (13). Nevertheless, the results of all three virion release assays indicate that the D(50)A<sub>CA</sub> mutant exhibits impaired virion release, while the P(1)A<sub>CA</sub> mutant does not. Furthermore, nonpolar substitutions of T(47)A<sub>CA</sub> compromise virion release more than polar substitutions at this position.

To determine whether the CA substitution mutants exhibited a gross defect in viral protein incorporation, 293T cells transfected with wild-type or mutant infectious viral DNA were metabolically labeled with [35S]methionine-cysteine. Virus lysates were immunoprecipitated with SIV antiserum and resolved by SDS-PAGE (Fig. 5C). In keeping with the virion release data (Fig. 5A and B), the G(2)A<sub>MA</sub> mutant exhibited a severe release defect. As envelope, RT, and integrase were detected for all of the other mutants, none of these substitutions caused a dramatic defect in viral protein incorporation.

Transmission EM phenotypes of wild-type and mutant SIVmac239. We investigated whether capsid morphology was affected by the CA substitutions by transmission EM of 293T cells transfected with the mutant panel. In the wild-type sample (Fig. 6A), we observed budding, immature, and mature virus particles, consistent with normal lentiviral morphology. All three typical mature core phenotypes were present in this sample: centric (core is round and centered with respect to the viral envelope), conical (core is cone-shaped and centered), and acentric (described above). No intracellular or extracellular viral particles were observed in the G(2)A<sub>MA</sub> sample (data not shown), a finding that is in keeping with EM analysis of G(2)A<sub>MA</sub> HIV-1 (14).

Substitution of alanine for HIV-1 D(51)A<sub>CA</sub> [HIV D(51)A<sub>CA</sub>] (51, 55) or leucine for HIV P(1)A<sub>CA</sub> [HIV P(1)L<sub>CA</sub>] (13) alters the core morphology of mature virions, such that the mature cores are predominantly acentric. The core morphology of substitution mutants of the SIV sequence homologues of either of these HIV-1 salt bridge participants has not, to our knowledge, been examined. Unlike P(1)L<sub>CA</sub> HIV (13), P(1)A<sub>CA</sub> SIV was morphologically wild type by EM (Fig. 6B). The differences between P(1)A<sub>CA</sub> SIV and P(1)L<sub>CA</sub> HIV are likely due to inherent differences in tolerance of substitutions at this position between HIV and SIV or to the fact that different substitutions were made. In contrast, consistent with the EM morphology of D(51)A<sub>CA</sub> HIV, D(50)A<sub>CA</sub> SIV virions exhibited gross morphological abnormalities (Fig. 6C and D). In this sample, several budding particles emerged from the same point in the plasma membrane, giving the buds an uncharacteristic multilobed shape (Fig. 6C). Importantly, there were some mature virions present in the D(50)A<sub>CA</sub> sample (Fig. 6F), but those also appeared to have mostly acentric cores. Some extracellular immature virions in the T(47)A<sub>CA</sub> photomicrographs were tethered...
FIG. 6. Transmission electron micrographs of wild-type and CA mutant SIVmac239 virions. 293T cells transfected with wild-type (WT) or mutant infectious viral DNA were harvested 1 day posttransfection, ultrathin sectioned, and analyzed by transmission EM. Labeled arrows indicate virions representative of typical virion morphologies: Ce, centric; I, immature; A, acentric; Co, conical. Unlabeled arrows show tethered immature virions (G), doublet and triplet particles (J), tethered virion chains (K), and accumulation of Gag at the plasma membrane (L). Bars, 100 nm.
together (Fig. 6G), similar to the tethered structures observed in late domain and deletion mutants of HIV-1 p6 (10, 21, 59).

In order to quantitatively determine whether T(47)ACA produces a higher proportion of acentric mature virions than wild-type virus, we compared the proportions of mature wild-type, D(50)ACA, and T(47)ACA SIVmac239 virions with acentric, centric, and conical mature cores (Table 1) by statistical analysis (see Materials and Methods). T(47)ACA virus had an intermediate proportion of acentric mature cores, which was significantly different from both the wild-type and D(50)ACA proportions. Therefore, the T(47)ACA substitution causes acentric virion core morphology, but to a lesser degree than the D(50)ACA substitution.

T(47)CCA had approximately wild-type proportions of budding, immature, and mature virions, but roughly 40% of the mature cores appeared to have condensed in an aberrant manner, as they had either two electron-dense core regions or acentric cores (Fig. 6H). Interestingly, the T(47)CCA mature core phenotype is similar to that reported for an HIV-1 p1-p6 cleavage site mutant (59). Few (~10%) mature virions were observed in the T(47)SCA sample, and the majority of these had abnormal or acentric cores (Fig. 6I). Approximately 90% of the virions in this sample were budding or extracellular and immature, and many of these virions were connected as doublet or triplet particles (Fig. 6J) or tethered to the plasma membrane in chains (Fig. 6K), a morphology that resembles both T(47)ACA and HIV-1 p6 mutants (10, 21, 59). No extracellular mature virions were found in cells transfected with the T(47)VCA mutant. Approximately 95% of Gag in this sample accumulated at the plasma membrane in discrete patches (Fig. 6L). Only 5% of the T(47)VCA virus particles were extracellular (data not shown), and the majority of these were large, multilobed, and immature, similar to the immature particles in the D(50)ACA sample.

Molecular modeling of SIVmac239 CA. As mentioned before, T(48)CA and D(51)CA form four hydrogen bonds in HIV CA (15) (see Fig. 7A for a model of these residues in HIV CA). Because both T(47)ACA and D(50)ACA had virion infectivity and release defects, and because the virion morphologies of these two mutants were so similar, we hypothesized that the interaction between HIV T(48)CA and HIV D(51)CA is conserved in SIV. To address this question, we constructed a model of SIVmac239 CA by homology to the crystal structure of the HIV-1 CA N terminus (15) using the program QUANTA (Fig. 7B). Due to sequence constraints, we limited this model to the region surrounding T(47)CA. Significantly, in the model, T(47)CA and D(50)CA form the same four hydrogen bonds that exist between the corresponding HIV CA residues: between the T(47)CA N and the D(50)CA O, between the D(50)CA N and the T(47)CA O, and between the T(47)CA O and the D(50)CA O.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Acentric mature cores</th>
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<tr>
<td>WT</td>
<td>46.6</td>
<td>0.002</td>
</tr>
<tr>
<td>D(50)A</td>
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<td>T(47)A</td>
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a P values were obtained by using a one-sided test for differences in the proportion of acentric cores relative to T(47)ACA SIVmac239. NA, not applicable.

DISCUSSION

In this study, we characterized the functional importance of hydrogen bonding at a conserved threonine in SIVmac239 CA, T(47)CA. Two observations from this study indicate that hy-
Hydrogen bonding at T(47)CA is essential to virus replication. First, mutants with nonpolar substitutions of T(47)CA were more compromised in virion infectivity and release than polar substitution mutants, indicating that hydrogen bonding at T(47)CA is specifically required for early and late events in the SIV life cycle. Secondly, T(47)VCA was severely deficient in virion release and infectivity relative to wild-type virus. As threonine and valine are quite similar from a space-filling perspective, the deficiencies of T(47)VCA are the result of the loss of hydrogen bonding at this position, rather than an alteration in the shape of the amino acid side chain.

The phenotypic similarities between the D(50)A, SIV CA mutant and T(47)CA substitution mutants [particularly the T(47)A, SIV CA mutant] indicate that these two residues likely have similar functionally important structural roles in SIV CA. Taken together, the results of the mutagenesis study and the modeling experiment presented herein strongly suggest that hydrogen bonding between SIV T(47)CA and D(50)CA is present in SIV CA and is required for viral replication. Because this interaction may be critical to CA function in the virus life cycle, these results provide a solid rationale for our ongoing efforts to determine the structure of mature SIV CA.

Two results indicate that T(47)CA SIV is phenotypically intermediate between D(50)A, SIV CA and WT SIV [i.e., substitution of D(50)CA is more detrimental to the virus than substitution of T(47)CA], suggesting that D(50)CA may have an additional important structural interaction in CA. First, the proportion of acentric mature cores observed in photomicrographs of T(47)CA is significantly different from that of either the wild type or the D(50)A, SIV CA mutant. Second, the budding deficiency of T(47)CA virus was not as extreme as that of D(50)A, SIV CA virus. One hypothesis to explain the intermediate phenotype of the T(47)CA mutant is that hydrogen bonding between T(47)CA and D(50)CA may position D(50)CA throughout the rearrangement of the SIV CA N terminus so that it can optimally participate in another requisite interaction, such as a salt bridge as in HIV.

The nuclear magnetic resonance structure of HIV-1 Pr55Gag from MA through the N terminus of CA was recently reported (50). In this structure, HIV-1 T(48)CA hydrogen bonds with the aspartate that participates in the salt bridge [HIV D(51)CA] in the immature form of CA, although only one of the four hydrogen bonds present in mature CA, the hydrogen bond between the D(51)CA N and the T(48)CA Oγ, likely exists in immature CA; the heavy atom distances for the other three bonds in the majority of the reported nuclear magnetic resonance models are greater than 3.5 Å. The fact that hydrogen bonding between T(48)CA and D(51)CA exists in both the immature and the mature forms of HIV CA supports the idea that T(48)CA positions D(51)CA for a requisite interaction with another residue [likely P(1)CA] throughout the rearrangement of the CA N terminus. However, based on the reported structures of mature and immature HIV CA (15, 50), it is reasonable to conclude that the pattern of hydrogen bonding between T(48)CA and D(51)CA changes subsequent to the proteolytic release of the HIV CA N terminus. As such, the three additional hydrogen bonds present only in mature HIV CA may provide critical support to the salt bridge in its largely hydrophobic environment.

We conclude that hydrogen bonding between the SIV homologues of HIV T(48)CA and D(51)CA is likely essential for proper CA function in virion release, infectivity, and core formation, suggesting that the interaction between these residues may be critical in HIV as well. If our conclusions about the requirement for hydrogen bonding between these two residues in SIV replication hold true in HIV, the change in hydrogen bonding between HIV T(48)CA and D(51)CA during the rearrangement of the CA N terminus may be a target for inhibitors of virus replication, since the structural constraints at these sites limit the acquisition of viable viral mutations.

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Under a licensing agreement between Bayer AG and the Johns Hopkins University, J.E.C. is entitled to a share of a payment received by the university on sales of products embodying the technology described in this article. The terms of this agreement are being managed by the Johns Hopkins University in accordance with its conflict-of-interest policies.

REFERENCES


