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Morphopoietic Determinants of HIV-1 Gag Particles Assembled in Baculovirus-Infected Cells

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The determinants for HIV-1 particle morphology were investigated using various deletion and insertion mutants of the Gag precursor protein (Gag) expressed in baculovirus-infected cells and ultrastructural analysis of membrane-enveloped Gag particles under the electron microscope. Five discrete regions were found to influence the size, the variability in dimension, and the sphericity of the particles: (i) the matrix (MA) N-terminal domain, within residues 10–21, the junctions of (ii) MA–CA (capsid), (iii) CA-spacer peptide SP1 and (iv) nucleocapsid (NC)–SP2, and (v) the p6 mature C-terminus. Internal regions (ii), (iii), and (iv) contained HIV-1 protease cleavage sites separating major structural domains. No particle assembly was observed for amb276, a MA–CA polyprotein mutant lacking the C-terminal third of the CA domain. However, MA–CA domains including the MHR (residues 277–306), or downstream sequence to CA residue 357, resulted in the assembly into tubular or filamentous structures, suggesting a helical symmetry of Gag packing. Mutant amb374, derived from amb357 by further addition of the heptadecapeptide motif 358HKARVLAEAMSQVTNSA374, overlapping the CA–SP1 junction and the SP1 domain, showed a drastic change in the pattern of Gag assembly, compared to amb357, with formation of spherical particles. These data suggested a novel function for the spacer domain SP1 acting as a spherical shape determinant of the Gag particle which would negatively affect the helical symmetry of assembly of the Gag precursor molecules conferred by the MHR and the downstream CA sequence, within residues 307–357.

Key Words: HIV-1; Gag precursor; assembly; morphological determinants; p6; SP1; SP2 domains; MA–CA junctions; helical symmetry; MV and CDV nucleoprotein core.

INTRODUCTION

The occurrence of virus-like particles in cells expressing solely the human immunodeficiency virus type 1 (HIV-1) Gag precursor (Pr55Gag) implied that this polyprotein contained all the sequence information required for its self-assembly into immature Gag particles, which, upon maturation, lead to infectious virions (Wills and Craven, 1991; Craven and Parent, 1996). Extensive mutagenesis of the retrovirus Gag structural domains, matrix (MA), capsid (CA), nucleocapsid (NC), and C-terminal p6 (reviewed in Boulanger and Jones, 1996; Craven and Parent, 1996; Kräusslich and Welker, 1996), and crystallographic analysis of MA (Hill et al., 1996; Massiah et al., 1994; Rao et al., 1995) and CA proteins (Gamble et al., 1996, 1997; Gitty et al., 1996; Kovari et al., 1997; Momany et al., 1996) have contributed to defining the regions in the Gag precursor which are essential for interaction and self-assembly of membrane-enveloped budding particles. In RSV, three discrete assembly domains have been identified, and termed M, for membrane-binding, I, for interaction, and L, for late step of budding (Craven and Parent, 1996). In HIV-1 Pr55Gag, M has been found to be confined to the N-myristoylated glycine and the N-terminal 31 residues of the MA domain, I has been mapped to the NC domain, and L at the N-terminal end of the p6 domain (Craven and Parent, 1996).

Virus particle morphogenesis has to be considered from a double point of view: (i) the quantitative aspect of the phenomenon, which can be assayed by the number of particles recovered from the extracellular medium, and its decrease or absence in the case of assembly-defective mutants; (ii) the qualitative analysis of the assembly process, which takes into account the parameters of the particles, i.e., their mean size, shape, and regular or irregular contours. In the case of HIV-1, however, the effects of gag mutations on particle assembly have mainly been studied in quantitative terms, and the numbers of Gag particles and mature infectious virions produced have been evaluated by CA immunonasays and RT enzymatic assays. By contrast, very little has been reported on the regions of the Gag precursor molecule and on the mechanisms which control the size, the shape, and the symmetry of the particles.

The present study describes the results of an ultrastructural analysis performed by electron microscopy (EM) on membrane-enveloped Gag particles assembled and released by baculovirus-infected insect cells expressing recombinant HIV-1 Gag precursor mutants. This system has the advantage of a high efficiency of Gag...
precursor production and particle assembly (Boulanger and Jones, 1996; Carrière et al., 1995; Chazal et al., 1994, 1995; Gheysen et al., 1989; Hughes et al., 1993; Luo et al., 1994; Overton et al., 1989; Royer et al., 1991, 1992); and provides Gag particle samples in sufficient amounts to obtain statistical data with reasonable confidence.

RESULTS

Electron micrographs showing WT and mutant Gag particles at the periphery of sectioned cells were observed and the parameters of the particles determined (Fig. 1 and Table 1). It was assumed that they represented nascent or "young" particles freshly released by budding from the plasma membrane. The results, exemplified in Figs. 2a, 2b, and 2c. Figure 2a presents the mean values for the external diameter of the mutant Gag particles. Their heterogeneity in size was estimated by the standard deviation (SD) of the mean values (Fig. 2b). Their propensity to assemble into nonspherical particles was estimated from the mean ratio of the minimum diameter (d) to the maximum diameter (D), defined as the sphericity index (d/D) (Fig. 2c and Table 2).

With the exception of mutant amb276, all the other mutants released extracellular Gag particles in significant numbers. The diameter of 59-released WT Gag particles was found to be 125.04 ± 12.45 nm (mean ± SD; n = 51; confidence interval = 3.5 nm at the P = 0.05 level), consistent with the previously reported dimensions of mature and immature HIV-1 particles (135±150 nm in diameter) observed by conventional transmission EM (Gelderblom, 1990). However, our recombinant WT Gag particles appeared to be rather homogeneous in size, a result which contrasted with the wide range of diameters, varying from 120 to 260 nm, for similar recombinant Gag particles analyzed by cryo-EM (Fuller et al., 1997). It is not possible to assess whether the homogeneity in size and shape of the WT Gag particles resulted from the artifactual effect of fixative and contrasting agents used in our EM study or, alternatively, whether the nascent, fibrin-included particles found in the vicinity of 519 cell plasma membrane would have been preserved in their original, native morphology. In the latter case, this would imply that heterogeneous Gag particles harvested from the culture medium and isolated by ultracentrifugation in velocity gradients for cryoEM analysis (Fuller et al., 1997), could have undergone some morphological alterations.

In contrast to WT Gag particles, more pleiomorphism was shown by Gag mutants, although to various degrees (Figs. 2c4). At the N-terminal extremity of the Gag precursor molecule, in the MA domain, the deletion dl10-21 (refer to Figs. 5c and 5d in Chazal et al., 1995), corresponding to the first α-helix H1, and the deletion dl101-143 (Fig. 3b), corresponding to the last α-helix H5 (Hill et al., 1996; Massiah et al., 1994) and the MAâ€CA junction, resulted in mutant particles significantly larger than the WT particles, with diameters of 206.01 ± 64.70 and 187.90 ± 3713 nm, respectively (Fig. 2a). The dl41-143 mutant (Fig. 3a) had a diameter of 165.82 ± 26.90 nm, intermediate between the WT particles and the dl10-21 and dl101-143 mutant particles. The dl10-21 mutant particles and, to a lesser degree, the dl41-143 and dl101-143 showed a higher heterogeneity in size and shape than the WT Gag particles, with a significant number of nonspherical particles adopting a dumbell shape (Figs. 2b, 2c, 3a, and 3b).

At the other extremity of the Gag precursor molecule, the deletion of the C-terminal half of p6<sup>gag</sup>, as in amb462, the deletion of the entire p6<sup>gag</sup> domain and most of the SP2 intermediate domain, as in amb438, or the deletion of p6<sup>gag</sup>, SP2, and the C-terminal end of the NC domain, as in amb426, had little or no effect on the final sizes of the mutant particles: 132.86 ± 9.19, 120.43 ± 10.02, and 127.04 ± 1113 nm, respectively (Fig. 2a). Likewise, partial or total deletion of the p6<sup>gag</sup> domain seemed to result only in minor changes in the degree of homogeneity and sphericity of mutant particles: a standard deviation of the mean value slightly lower than that for WT was observed for amb462 (9 nm vs 12 nm for WT; Fig. 2b) and a slightly higher sphericity index for amb438 (0.93 ± 0.06 vs 0.89 ± 0.01 for WT; Fig. 2c and Table 2). In contrast to these deletions, the fusion of an extra retroviral sequence to the carboxy-terminal extremity of p6<sup>gag</sup>, as in GagPR67, apparently affected the particle size and homogeneity to a greater extent. GagPR67, which contains a p6<sup>gag</sup>-fused protease (PR) domain inactivated by a D33G mutation at its active site, released irregular particles, varying in shape from small spherical particles to elongated structures (Royer et al., 1997). Their mean diameter was found to be slightly smaller than that of WT particles (m = 109 nm; Fig. 2a), but their heterogeneity (SD = 77.32 nm) and lack of sphericity (d/D = 0.79) were both higher (Figs. 2b and 2c). Further addition of portions of the reverse transcriptase domain (Pol) at the C-terminus of Gag polyprotein, as in GagPRPol84 or GagPRPol112 constructs (Royer et al., 1997), abolished assembly and budding (not shown).

Since the quantitative changes occurring with p6 mutations were rather discrete, in comparison to carboxytruncated mutants such amb333 or amb341, the Student's test was applied to the parameters of the WT, amb462, amb438, and GagPR67 particle populations. The difference in particle heterogeneity was apparently significant at the P = 0.05 level between WT and amb462 (t = 3.25, P = 0.00158), as well as between WT and GagPR67 (t = 3.73, P = 0.001), and the difference in sphericity index between the populations of WT and amb438 particles was also significant (t = 4.65, P = 0.001). This suggested that the carboxy terminus of Pr55gag would play some
subtle but significant conformational role in the Gag particle morphogenetic process. The processing of HIV-1 Pr55Gag by the virus-coded proteinase (PR) results in the release of four major structural domains, MA, CA, NC, and p6gag, along with two spacer peptides delineated by additional cleavage sites at the CA–NC junction (position 363–364) and the NC-p6gag junction (position 433–434), respectively (Craven et al., 1995).
Characteristics of Recombinant Gag Precursor Mutants and Mutant Gag Particles

<table>
<thead>
<tr>
<th>Gag clone designation</th>
<th>Mutant sequence</th>
<th>Domains involved</th>
<th>Sample size</th>
<th>Particle morphology</th>
<th>Postulated symmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Pr55Gag</td>
<td>D</td>
<td>D</td>
<td>51</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>di10-21</td>
<td>-G²⁰[L²¹]</td>
<td>MA (E²¹)</td>
<td>55</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>di43-143</td>
<td>-E²⁰ [NSR]</td>
<td>MA, MA/CA</td>
<td>57</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>di101-143</td>
<td>-A²⁰ [GN]</td>
<td>MA/CA</td>
<td>48</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>amb276</td>
<td>-M²⁷[stop]</td>
<td>CA (-HMR)¹</td>
<td>0</td>
<td>No particle</td>
<td>D</td>
</tr>
<tr>
<td>amb306</td>
<td>-A²⁰[stop]</td>
<td>CA (+HMR)¹</td>
<td>18</td>
<td>Tubular</td>
<td>H</td>
</tr>
<tr>
<td>amb333</td>
<td>-[33][FWNSSLD]-stop</td>
<td>CA</td>
<td>16</td>
<td>Tubular</td>
<td>H</td>
</tr>
<tr>
<td>amb341</td>
<td>-A²⁴[ILY]-stop</td>
<td>CA</td>
<td>30</td>
<td>Tubular</td>
<td>H</td>
</tr>
<tr>
<td>amb357</td>
<td>-G²⁵[WNSSLD]-stop</td>
<td>CA/SP1</td>
<td>21</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>amb374</td>
<td>-A²⁷[GIY]-stop</td>
<td>SP1</td>
<td>24</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>amb426</td>
<td>-C²⁶[LEF]-stop</td>
<td>NC</td>
<td>24</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>amb438</td>
<td>-W²⁵[LEF]-stop</td>
<td>SP2</td>
<td>50</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>amb462</td>
<td>-S²⁶[WNSSLD]-stop</td>
<td>p6¹⁰¹²</td>
<td>48</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>in357</td>
<td>-G²⁵[WNSS]</td>
<td>CA/SP1</td>
<td>10</td>
<td>Spherical and tubular</td>
<td>SS, H</td>
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<td>SP1</td>
<td>89</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>in438</td>
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<td>SP2</td>
<td>16</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>di427-438</td>
<td>-C²⁶[LEF]P</td>
<td>NC/SP2</td>
<td>47</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>di437-444</td>
<td>-K²⁶[ILP²⁴⁴]-stop</td>
<td>SP2</td>
<td>62</td>
<td>Spherical</td>
<td>SS</td>
</tr>
<tr>
<td>GagPR67</td>
<td>GagPr55-PRD33C¹</td>
<td>p6¹⁰¹²//PR(11kDa)</td>
<td>24</td>
<td>Spherical and tubular</td>
<td>SS, H</td>
</tr>
</tbody>
</table>

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¹ The sequences are written in single-letter code amino acids, and amino acids inserted at cloning cassettes are in brackets.
² The respective positions of the different structural domains of HIV-1 LAI Pr55Gag are indicated in Fig. 1. For the MA mutant di10-21, the short deleted sequence (residues 11±20) is shown in parenthesis. Double slash bars indicate the junction between structural domains.
³ Sample size represents the number of particles (n) observed under the EM and quantitated, as shown in Fig. 2.
⁴ Assembly of Gag molecules has been shown not to follow the icosahedral symmetry, but a mechanism of interaction of membrane-bound semispherical sectors (SS) (Fuller et al., 1997). Tubular filamentous particles are assumed to be tubular (h) symmetry.
⁵ Deletion involving (−) or not (+) the major homology region (MHR; Wills and Craven, 1991), located within residues 285–305 in HIV-1 Pr55Gag.
⁶ Addition of the active site-inactivated protease (PR) domain (D to G substitution; Royer et al., 1997) to the C-terminus of p6¹⁰¹².

and Parent, 1996; Henderson et al., 1992). The role of SP1 (residues 364±377) and SP2 (residues 434±447) in Gag particle morphogenesis was also analyzed by EM, using Pr55Gag carrying mutations in (or near) these two intermediate domains. Mutants di437-444 (SP2-deleted; Fig. 3c), di427-438, carrying a deletion involving the NC-SP2 junction (Fig. 3d), and the SP2 insertion mutant in338 showed Gag particles with a mean diameter greater than that of WT particles (145.63, 152.60, and 145.81 nm, respectively), with a significantly higher dispersion of the values (SD ranging from 22 to 36 nm). For the two deletion mutants, but not for the insertion mutant in338, there was a lower sphericity index (0.77 and 0.80), compared to WT particles. Two insertion mutants, in374, which carried an insertion within the SP1 domain, and in357, carrying an insertion at the CA-SP1 junction (Table 1), assembled particles slightly larger than those of the WT (136.94 and 129.84 nm, respectively). However, in357 Gag particles appeared to be highly pleiomorphic, with a SD value of 48 nm, and showed many irregular and tubular structures which lowered their sphericity index to 0.77. These data suggested that the spacer domains SP2 and SP1 play a significant role in the Gag particle morphology. The importance of SP1 and the CA-SP1 junction was confirmed by EM analysis of Gag carboxy-truncation mutants.

Carboxy-truncated Gag polyproteins with extensive deletions from the C-terminus were studied in EM for size and shape characteristics. Mutant amb276, deleted of p6, SP2, NC, SP1, and the C-terminal third of CA, including the major homology region (MHR; Wills and Craven, 1991; Craven and Parent, 1996), failed to assemble any Gag particle. However, amb306, which failed to form spherical particles, assembled long tubular structures of 15 to 93 nm in diameter (60 ± 27 nm; m ± SD) and heterogeneous in length, up to 8 µm (Figs. 2a and 2d). Similar tubular assemblies of heterogeneous lengths protruding from the plasma membrane were observed with amb333, amb341, and amb357 (Figs. 4b±4f), although they showed less heterogeneity in diameter (32 ± 4, 48 ± 5, and 45 ± 15 nm, respectively) than amb306. These tubular structures were labeled with anti-CA monoclonal antibody and immunogold-labeled conjugate (Fig. 4e), suggesting that the protein layer underneath the membrane lipid bilayer consisted of Gag precursor molecules. All the other Gag mutants carrying smaller carboxy-terminal deletions, amb374 (lacking NC, SP2, and p6¹⁰¹²; Fig. 4g) amb426, amb438 (lacking SP2 and p6¹⁰¹²), and amb462 (deleted of the p6¹⁰¹² C-terminal half), assembled spherical particles (not shown; refer to Carrière et al., 1995).
The regions of Pr55Gag which influenced the overall particle size, their variability in size and their degree of sphericity were found to be located at five discrete regions of the molecule: (i) at the N-terminus of the MA, within residues 10–21, (ii) at the MA–CA junction, (iii) at the CA–SP1 junction, (iv) at the NC–SP2 junction, and (v) at the p6\textsuperscript{gag} C-terminus. It is noteworthy that three of these regions correspond to HIV-1 protease cleavage sites, suggesting that they represent accessible and flexible hinges between structural domains. It is reasonable to conceive that these hinges could have significant morphopoietic effects on the Gag particles.

The central portion of the MA domain has been shown to be essential for production of infectious virions and assembly of recombinant Gag particles (Chazal et al., 1995; Freed et al., 1994; Yu et al., 1992). Its two bounding domains, the H1 α-helix, which was mutated in dl10-21, and which has been assigned to bind to plasma mem-
FIG. 4. Electron microscopy (a–d, f, g) and immunoelectron microscopy (e) of Gag particles assembled by Gag carboxy-truncated mutants (amber mutation, amb) at the plasma membrane of Sf9 cells. CA deletion mutants amb306 (a), amb333 (b), amb341 (c), amb357 (d–f), and amb374 (g). In (e), cell section was reacted with anti-CA mAb and 5-nm gold-labeled anti-mouse IgG conjugate. Bar, 100 nm.
TABLE 2

Degree of Sphericity of WT and Mutant Gag Particles

<table>
<thead>
<tr>
<th>Clone</th>
<th>d:D ratio (mean)</th>
<th>± SD</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Pr55Gag</td>
<td>0.89</td>
<td>0.01</td>
<td>51</td>
</tr>
<tr>
<td>dl10-21</td>
<td>0.65</td>
<td>0.20</td>
<td>55</td>
</tr>
<tr>
<td>dl41-143</td>
<td>0.85</td>
<td>0.12</td>
<td>57</td>
</tr>
<tr>
<td>dl101-143</td>
<td>0.67</td>
<td>0.21</td>
<td>48</td>
</tr>
<tr>
<td>amb306</td>
<td>0.01</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>amb333</td>
<td>0.00</td>
<td>NA</td>
<td>16</td>
</tr>
<tr>
<td>amb341</td>
<td>0.01</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>amb357</td>
<td>0.01</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>amb374</td>
<td>0.87</td>
<td>0.09</td>
<td>24</td>
</tr>
<tr>
<td>amb426</td>
<td>0.91</td>
<td>0.08</td>
<td>24</td>
</tr>
<tr>
<td>amb438</td>
<td>0.93</td>
<td>0.06</td>
<td>50</td>
</tr>
<tr>
<td>amb462</td>
<td>0.89</td>
<td>0.07</td>
<td>48</td>
</tr>
<tr>
<td>in357</td>
<td>0.77</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>in374</td>
<td>0.87</td>
<td>0.08</td>
<td>89</td>
</tr>
<tr>
<td>in438</td>
<td>0.92</td>
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<td>16</td>
</tr>
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<td>dl427-438</td>
<td>0.80</td>
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<td>47</td>
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<tr>
<td>dl437-444</td>
<td>0.77</td>
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<td>62</td>
</tr>
<tr>
<td>GagPr67</td>
<td>0.79</td>
<td>0.20</td>
<td>18</td>
</tr>
</tbody>
</table>

* a The sphericity index of Gag particles was estimated from the mean ratio of the minimal (d) to the maximal (D) diameter. The histogram is shown in Fig. 2c. The sample size (n) represents the number of particles quantitated. SD, standard deviation of the mean.

* b NA, not applicable (tubular or filamentous particles).

brane phospholipids (Kräusslich and Welker, 1996), and the C-terminal portion of the MA, overlapping the second basic signal and the MA–CA junction, mutated in dl101-
143, apparently contain major morphological determinants, influencing the particle size and shape. At the C-terminus of the Gag precursor molecule, partial or total deletion of the p6<sup>306</sup> domain resulted in a slight, albeit statistically significant, increase in particle homogeneity and sphericity (as in amb462 and amb438), whereas the addition of an extra retroviral sequence to the C-terminus of p6<sup>341</sup> (as in GagPr67) had the reverse effect. This would imply that the p6<sup>341</sup> domain would also have some influence on Gag conformation and assembly, as previously suggested (Hughes et al., 1993; Royer et al., 1997).

Our results with amb276 and amb306 suggested that a recombinant HIV-1 Gag precursor consisting of the MA and carboxy-truncated CA domains was not capable of self-assembly in vivo without the MHR sequence (residues 285±305; Wills and Craven, 1991; Craven and Parent, 1996), confirming previous studies on HIV-1 and other retroviruses (Carrière et al., 1995; Craven et al., 1995; Mammano et al., 1994; Strambio-de-Castilla and Hunter, 1992). The addition of a CA sequence comprising the MHR brought enough assembly information to allow the formation of cylindrical or filamentous Gag particles. These types of structures suggested a helical symmetry of Gag packing. Further extension of the CA domain within residues 307 to 357, as in amb333, amb341, and amb357 which still assembled filamentous particles, implied that the CA sequence within residues 307±357 did not influence the helical symmetry of HIV-1 Gag packing already conferred by the MHR.

The role of Gag spacer peptides in retrovirus particle assembly and infectious virus production has been already investigated in RSV and HIV. The peptide interspacing the CA and NC domains of RSV Gag precursor, although having no significant sequence homology with HIV SP1, has been found to be required for forming regular-shaped infectious virions (Craven et al., 1993; Krishna et al., 1998). Likewise in HIV-1, deletions of SP1 or mutations at the CA±SP1 junction abolished infectivity (Goettlinger et al., 1989; Kräusslich et al., 1995; Pettit et al., 1994). The integrity of SP1 and the CA±SP1 junction has been found to be crucial for viral particle formation in HIV-1 (Accola et al., 1998), and the deletion of a proline-rich region at the CA±NC junction of recombinant HIV-2 Gag precursor (within residues 372±377) abolished particle assembly (Luo et al., 1994). Viral particles released by COS 7 cells transfected by a SP1-deleted HIV-1 provirus have been found to contain a tube-shaped electron-dense core (Kräusslich et al., 1995). It has been suggested that SP1 deletion had a deleterious effect on the sequential processing of Gag polyprotein (Pettit et al., 1994) and ordered assembly of the virions (Kräusslich et al., 1995). Our observation that amb357 assembled Gag tubular structures confirmed previous studies using C-truncated mutants of HIV-1 Gag (Gheysen et al., 1989; Goettlinger et al., 1989; Hockley et al., 1994; Loyett et al., 1992) and point mutants of the CA domain of M-PMV Gag precursor (Strambio-de-Castilla and Hunter, 1992). These tubes were also reminiscent of the elongated structures obtained in experiments of in vitro assembly of CA±NC domains of RSV and HIV (Campbell and Vogt, 1995) and of HIV-1 recombinant CA protein (Ehrlich et al., 1992; Gross et al., 1997).

Full-length recombinant Gag precursor and immature HIV-1 particles have recently been shown to lack an icosahedral organization, as previously hypothesized (Nermut and Hockley, 1996), and to assemble via accretion of membrane-bound semispherical sectors (Fuller et al., 1997). Furthermore, crystals of RSV CA protein show a helical arrangement of protein subunits (Kovari et al., 1997). The addition to the amb357 sequence of a short stretch of amino acids from the CA-SP1 domains, overlapping the CA carboxy-terminal hexapeptide (residues 358±363) and almost the entire SP1 (viz. 11 residues of the SP1 tetradecapeptide), to generate amb374, resulted in the assembly of Gag spherical particles (compare panels f and g in Fig. 4). Although smaller than WT Gag particles (103.66 ± 16.98 nm, n = 24, confidence interval of 114 nm at the P = 0.05 level), the amb374 particles were almost as homogeneous in size as WT particles and presented a similar sphericity index (0.87; Fig. 2c; Table 2). The modification of the phenotype of Gag particles, from a tubular (as for amb306, amb333, amb341, and amb357) to a spherical shape (as for amb374), suggested that the addition of the CA±SP1 junc-
tion and SP1 peptide to the MA±CA domains of HIV-1 was sufficient to alter the helical symmetry of assembly of the Gag molecules.

However, recombinant HIV-1 CA protein has been found to self-assemble in vitro as tubules, regardless of the presence of SP1 at the C-terminus (Gross et al., 1997). Their diameter, 35 nm, is similar to the value found for our amb357 mutant filamentous particles (45 ± 15 nm; Figs. 2a and 2b). This would confirm that the peptide domain conferring the helical symmetry of Gag packing in vitro and in vivo is localized within the CA, but would imply that the morphopoietic function assigned to SP1 would take place only in vivo at the budding sites, in the presence of the MA domain, and in the plasma membrane environment. Our observation therefore suggests a novel function for the SP1 domain and the CA±SP1 junction of HIV-1, acting as a spherical shape determinant of the immature Gag particle in vivo and as a silencer of the helical symmetry of MA±CA assembly, conferred by the MHR and the downstream CA silencer of the helical symmetry of MA–CA assembly, particle phenotype was the peptide motif 358HKARVLAE–1994).

The minimum sequence required to transform the Gag particle phenotype was the peptide motif 358HKARVLAE-AMSQVTNSA 37 4, which differentiates amb357 from amb374. This peptide was compared to sequences deposited in databanks using the FASTA program. It was found to present 52% homology within a 14-residue overlap and 66% homology within a 9-residue overlap, with the sequence KPRIAEICDIDN(T) found in the nucleocapsid proteins (residues 238±250) of two paramyxoviruses, human measles virus (MV), and canine distemper virus (CDV) (Barrett and Mahy, 1984; Rozenblatt et al., 1985). The CDV and MV nucleocapsid proteins interact with the M proteins during virion formation and have been postulated to confer the helical symmetry to the nucleoprotein core of the virus. Both Gag CA±SP1 junction (Accola et al., 1998) and MV±CDV nucleoprotein homologous peptide have a high probability of adopting an α-helix conformation within the context of the whole protein (data not shown). It would be rewarding to determine whether the conserved 14-mer peptide motif has any morphopoietic function in a viral protein context different from HIV-1 Gag and represents per se a specific determinant of the symmetry of the CDV and MV cores.

MATERIALS AND METHODS

Gag constructs

The construction of the recombinant baculoviruses expressing the WT Pr55Gag and most of the mutants used in this study has been described in detail elsewhere (Carrière et al., 1995; Chazal et al., 1995; Royer et al., 1991, 1992). Carboxy-truncated mutant amb462 has been deleted of the C-terminal half of the p6 domain, amb438 of the entire p6 and the C-terminal half of the SP2 intermediate domain, amb426 of the p6 and SP2 domains of the C-terminal region of the NC downstream to the second zinc finger, amb374 has been deleted of the entire NC, and amb357 of SP1 and NC. Further carboxy truncations within the CA domain have generated mutants amb341, amb333, amb306, and amb276 (Carrière et al., 1995). The internal deletion in mutant dl437-444 (residues 437IWPSYKGR444) removed most of the SP2 domain (434LGKIWPSYKGRPGNF448) (Huvent et al., 1998). Deletion in mutant dl427-438 involved the NC–SP2 junction, with the sequence 426C–TERQANF±LGK1WP439 replaced by 426C-[LEFQ]-P439 (Huvent et al., 1998). The MA deletion mutant dl10-21 has been characterized in a previous study (Chazal et al., 1995). For MA mutant dl141-143, the 5′ gag sequence, coding for the MA N-terminal domain, was cut at the Alul site at codon position 40 and ligated to the 3′ moiety of in143 gag, cut, and blunted at the unique EcoRI site present in its insertion linker (Chazal et al., 1994). For mutant dl101-143, the gag 5′ sequence of the insertion mutant in100 was ligated to the 3′ moiety of in143 gag, both cut and blunted at their unique EcoRI site (Chazal et al., 1994). The abbreviated names and sequence characteristics of the mutants used in this study are summarized in Table 1, and a schematic drawing of the Gag precursor domains depicting the position and extent of the various mutations is shown in Fig. 1.

Ultrastructural analysis

Sf9 cells were infected by recombinant baculovirus at a M.O.I. of 5 PFU/cell, and cells expressing the different Gag constructs were harvested at 48 h after infection. To retain the maximum number of budding Gag particles at the periphery of the cells, fibrinogen was added to the cell suspension (5 mg/ml in Tris-buffered saline; TBS), and clotting of fibrin within the cell pellet was induced by addition of thrombin (100 μg/ml in 01% CaCl2 in H2O) immediately before low-speed centrifugation of the cells. Cell pellets were then processed for EM analysis as previously described (Carrière et al., 1995). Several cell sections per each Gag mutant were examined under the Hitachi-H7100 electron microscope. Except for some low Gag particle producers, the parameters were usually determined on 40 to 60 Gag particles per mutant.

For immunoelectron microscopy (IEM), thin sections were pretreated with a saturated aqueous solution of sodium metaperiodate for 10 min, followed by a rinse in H2O, and 10 min in 01 N HCl. After etching, the grids were washed in H2O for 5 min and incubated on drops of TBS supplemented with 0.05% Tween 20 (TBS-T), 0.5%
cold water fish skin gelatin (Sigma), 0.5% nonimmune goat serum, 0.5% bovine serum albumin, and the required antibody (Bendayan and Zollinger, 1983). Incubation with the primary anti-CA monoclonal antibody (Epilcclone 5001; diluted to 1:100) was carried at 4°C overnight (Carrière et al., 1995), followed by reaction with secondary 5-nm colloidal gold-labeled anti-mouse IgG antibody (Amersham; diluted to 1:10) at room temperature for 1 h.

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