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COMMUNICATION

HIV-1 Gag Extension: Conformational Changes Require Simultaneous Interaction with Membrane and Nucleic Acid

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The retroviral Gag polyprotein mediates viral assembly. The Gag protein has been shown to interact with other Gag proteins, with the viral RNA, and with the cell membrane during the assembly process. Intrinsically disordered regions linking ordered domains make characterization of the protein structure difficult. Through small-angle scattering and molecular modeling, we have previously shown that monomeric human immunodeficiency virus type 1 (HIV-1) Gag protein in solution adopts compact conformations. However, cryo-electron microscopic analysis of immature virions shows that in these particles, HIV-1 Gag protein molecules are rod shaped. These differing results imply that large changes in Gag conformation are possible and may be required for viral formation. By recapitulating key interactions in the assembly process and characterizing the Gag protein using neutron scattering, we have identified interactions capable of reversibly extending the Gag protein. In addition, we demonstrate advanced applications of neutron reflectivity in resolving Gag conformations on a membrane. Several kinds of evidence show that basic residues found on the distal N- and C-terminal domains enable both ends of Gag to bind to either membranes or nucleic acid. These results, together with other published observations, suggest that simultaneous interactions of an HIV-1 Gag molecule with all three components (protein, nucleic acid, and membrane) are required for full extension of the protein.

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Abbreviations used: HIV-1, human immunodeficiency virus type 1; MA, matrix; CA, capsid; NC, nucleocapsid; NA, nucleic acid; WT, wild type; VLP, virus-like particle; SANS, small-angle neutron scattering; EM, electron microscopy; ssDNA, single-stranded DNA; tBLM, tethered bilayer lipid membrane; NR, neutron reflectivity; nSLD, neutron scattering length density.

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Introduction

Expression of a single retroviral protein, termed Gag, is sufficient for virus particle formation in mammalian cells.[1,2] Gag is a multi-domain protein always containing, from N- to C-terminus, a matrix (MA) domain, a capsid (CA) domain, and a nucleocapsid (NC) domain. The particles assembled from Gag are immature virions; in most retroviruses, including lentiviruses such as human immunodeficiency virus type 1 (HIV-1), these particles form as Gag accumulates at the cytoplasmic face of the plasma membrane. Targeting of Gag to the plasma membrane is a function of the MA domain and involves, on the one hand, an electrostatic interaction between basic residues in MA and anionic lipids in the membrane and, on the other hand, a hydrophobic interaction between membrane lipids and myristic acid, a 14-carbon saturated fatty acid at the extreme N-terminus of the protein.[3] The protein–protein interactions leading to virus assembly are largely or exclusively a function of the CA domain, while the NC domain plays a principal role in the interactions of Gag with nucleic acids (NAs).

Immature retrovirus particles are roughly spherical, with an average diameter of around 1100 Å. The Gag proteins in these particles are extended rods, approximately 200 Å long.[4] They are arranged as radii of the particles, with their N-termini in contact with the lipid bilayer that surrounds the particle and their C-termini projecting into the interior of the particle, presumably in contact with RNA. A transmission electron micrograph of these particles, budding from the plasma membrane of a mammalian cell, is shown in Fig. 1a.

We have previously characterized the properties of recombinant HIV-1 Gag protein, purified from bacteria.[7] This protein differs from authentic, wild-type (WT) HIV-1 Gag protein in lacking both the myristic acid modification at its N-terminus and a domain at the extreme C-terminus termed p6. When NA is added, this protein assembles into virus-like particles (VLPs); however, these VLPs are far smaller than authentic retrovirus particles, with a diameter of only 250 Å to 300 Å.[5] Figure 1b shows examples of the small VLPs formed with NA alone. These VLPs are too small to be composed of 200-Å rods of Gag. In fact, the shell of protein forming these VLPs appears to be only 70 Å to 80 Å thick. However, correctly sized VLPs are assembled if inositol pentakisphosphate as well as NA is added to purified Gag.[9] The striking difference between the particles in Fig. 1a and b implies that HIV-1 Gag can adopt alternative conformations, one extended (forming authentic particles) and one bent (as in the VLPs in Fig. 1b).

This conformational freedom presumably reflects the flexibility of the linker regions between the domains in the Gag polyprotein. Solution-state NMR demonstrated the MA and CA domains to be rotationally uncoupled by a 26-amino-acid linker.[10] Structural determination of the CA domain by crystallography revealed five disordered residues linking separate N-terminal and C-terminal domains.[11] Furthermore, NC, p6, and p2 domains appear to be largely unstructured, except for the Zn-finger regions within NC.[12–15] Difficulties in characterizing intrinsically disordered domains have led to a lack of high-resolution information for the intact Gag protein.

In previous investigations, structural properties of recombinant Gag protein in solution were probed using hydrodynamic data and small-angle neutron scattering (SANS) together with molecular modeling.[7] This study used a Gag mutant (designated WM Gag) that was inhibited from dimerizing. The results indicated that the protein is compact in solution, with its terminal domains situated close in three-dimensional space. It seems likely that the protein monomer in solution actually adopts an ensemble of interconverting, relatively compact structures.

In authentic immature particles, each Gag molecule is in contact with other Gag molecules, with the lipid bilayer surrounding the particle, and with the RNA within the particle. The question then arises as to which of these interactions is responsible for the extension of the Gag protein in these particles. In the present work, we have explored the conditions under which HIV-1 Gag protein is compact, as it is in free solution and small VLPs, or extended, as it is in immature virions. We used neutron scattering methods to dissect the contributions of protein–protein, protein–lipid, and protein–NA interactions to the extension of the protein.

Protein–protein interactions

HIV-1 Gag exists in solution in monomer–dimer equilibrium.[6] The interface mediating the dimeric interaction is in the C-terminal portion of the CA domain. In order to determine whether dimerization leads to extension of the WT protein, we used SANS to measure the mean scattering particle size or radius of gyration, R_g, of WT Gag over a broad concentration range. Results of this experiment are shown in Fig. 1c. The figure shows the R_g data (left axis) for WT Gag and WM Gag as a function of protein concentration; for the WT Gag, the fraction of protein present in dimers is depicted with a blue broken line and was calculated using a K_d of 3.9 μM in D_2O buffer.[6]

As shown in Fig. 1c, the R_g of the WT Gag protein increased as the dimeric fraction increased. At low concentration (0.25 mg/ml, or 5 μM), where 55% of the molecules are in dimers, the weight-averaged R_g is 38 Å, slightly larger than the monomeric Gag value of 35 Å. As the protein concentration increases, the