Membrane Interactions of the Mason-Pfizer Monkey Virus Matrix Protein and Its Budding Deficient Mutants

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Abstract

Matrix proteins (MAs) play a key role in the transport of retroviral proteins inside infected cells and in the interaction with cellular membranes. In most retroviruses, retroviral MAs are N-terminally myristoylated. This modification serves as a membrane targeting signal and also as an anchor for membrane interaction.

The aim of this work was to characterize the interactions anchoring retroviral MA at the plasma membrane of infected cell. To address this issue, we compared the structures and membrane affinity of the Mason-Pfizer monkey virus (M-PMV) wild-type MA with its two budding deficient double mutants, that is, T41I/T78I and Y28F/Y67F. The structures of the mutants were determined using solution NMR spectroscopy, and their interactions with water-soluble phospholipids were studied. Water-soluble phospholipids are widely used models for studying membrane interactions by solution NMR spectroscopy. However, this approach might lead to artificial results due to unnatural hydrophobic interactions. Therefore, we used a new approach based on the measurement of the loss of the ^{1}H NMR signal intensity of the protein sample induced by the addition of the liposomes containing phospholipids with naturally long fatty acids. HIV-1 MA was used as a positive control because its ability to interact with liposomes has already been described. We found that in contrast to HIV-1, the M-PMV MA interacted with the liposomes differently and much weaker. In our *in vivo* experiments, the M-PMV MA did not co-localize with lipid rafts. Therefore, we concluded that M-PMV might adopt a different membrane binding mechanism than HIV-1.

Introduction

Mason-Pfizer monkey virus (M-PMV) is a simple non-transforming retrovirus, which causes immunodeficiency of infected animals. M-PMV is the most thoroughly understood *Betaretrovirus* [1]. Since the formation of immature virus particles and their membrane association and budding are spatially and temporally separated, M-PMV serves as an excellent model for the study of the late phases of retroviral life cycle.

The matrix protein (MA), the N-terminal part of the main structural polyprotein Gag, is crucial for membrane targeting of immature virus particles and their interactions with the plasma membrane [2,3]. MAs of most retroviruses are myristoylated and structurally highly similar [4]. The HIV-1 MA interacts with the plasma membrane through a bipartite signal consisting of a patch of basic residues and a myristoyl residue. The latter is exposed upon interaction with the membrane by a mechanism called the myristoyl switch [5]. It is induced by the interaction of Gag with phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2], a phospholipid occurring exclusively in the plasma membrane [6,7]. The HIV-1 MA has the highest binding affinity for PI(4,5)P_2, even though it also interacts with phosphatidylinositol-3,5-bisphophate; phosphatidylinositol-3,4,5-trisphophate; phosphatidylserine (PS); phosphatidylethanolamine; and phosphatidylcholine (PCH) [8,9]. The interaction with PS, phosphatidylethanolamine,

and PCH was observed also for the non-myristoylated HIV-1 MA, indicating that the myristoylation is dispensable for this process [9].

The interaction of M-PMV MA with membrane phospholipids has been so far investigated for differently phosphorylated phosphatidylinositolphosphates, and no specific interaction with PI(4,5)P_2 was found [10]. Contrary to HIV-1, only minor changes in chemical shifts of the M-PMV MA protein were observed upon its interaction with dibutanoyl-PI(4,5)P_2, indicating a much weaker binding affinity [10]. A binding affinity comparable to that for HIV-1 MA was achieved when dioctanoyl-PI(4,5)P_2 [diC8-PI(4,5)P_2] was used [10]. It was also shown that only the myristoylated M-PMV MA interacted with diC8-PI(4,5)P_2 in contrast to the non-myristoylated MA [10].

Over the years, there have been numerous M-PMV MA mutants constructed with impaired virus stability, site of assembly, release, infectivity, or membrane association [11–14]. One of the particularly interesting mutations was T41I/T78I, which was found to reduce infectivity by blocking the budding capability of the immature virus to about 60% of the wild type (WT) [11]. In subsequent work, Stansell *et al.* proposed that the budding arrest of the T41I/T78I mutant was caused by stronger hydrophobic interactions of the isoleucine residues with the N-terminal myristoyl [15]. To test this hypothesis, they constructed several mutants in which tyrosine residues were exchanged for phenylalanines [15]. The authors identified a double mutant Y28F/Y67F that exhibited normal intracellular transport, but the virus release was even lower than that of the T41I/T78I mutant, which supported their initial hypothesis [15].

In this work, we investigated the binding of M-PMV MA to water-soluble phospholipids and liposomes of different compositions and compared it with the binding of HIV-1 MA. We also investigated structural perturbations caused by T41I/T78I and Y28F/Y67F mutations and the effect of the mutations on the binding of MA to liposomes in order to explain their phenotype, and we performed computer simulations of myristoyl unbinding from M-PMV MA WT and mutant MAs to test the hypothesis that the binding decrease is caused by changed hydrophobicity of the protein core. To support the results from NMR experiments, we also performed *in vivo* experiments in two cell lines (HEK293 and COS-1) to compare M-PMV and HIV-1 immature viral particles binding with cell membrane lipid rafts.

Results

Structures of the myristoylated mutants of the M-PMV MA

Using NMR spectroscopy, the structures of the myristoylated T41I/T78I and Y28F/Y67F mutants of M-PMV MA were determined. The proteins were prepared with C-terminal extension of 18 aa from the phosphoprotein and a hexahistidine-tag. Previously, we have shown that the phosphoprotein-hexahistidine-tag extension has no effect on the structure of WT M-PMV MA [10]. The selective cleavage of non-myristoylated MAPPHis by M-PMV protease (Pr13) was used to separate the myristoylated MA protein from its non-myristoylated form [16]. This approach was applied also for the T41I/T78I and Y28F/Y67F mutants. The resonance assignments of both myristoylated mutants were deposited into Biological Magnetic Resonance Data Bank (T41I/T78I-BMRB ID: 34015 and Y28F/Y67F-BMRB ID: 25087) [17]. Moreover, the resonance assignment of the Y28F/Y67F mutant was published separately [18]. Structure calculations were based mainly on nuclear Overhauser effect (NOE) distance constraints and dihedral angle restrictions calculated with TALOS + software [19]. The position of the myristoyl group was determined from the contacts between the myristoyl and the amino acids of the protein using the 3D ^{13}C-edited/^{13}C-filtered NOE spectroscopy (NOESY) data [20]. The best 40 structures of the Y28F/Y67F mutant were chosen based on the quality of their structural parameters and were deposited into the Protein Data Bank (PDB) database (PDB ID: 2MV4).

Due to the high structural similarity of the M-PMV WT MA and the T41I/T78I mutant, residual dipolar couplings (RDCs) were used in the later stage of the structure calculation to re-refine the WT and T41I/T78I MA mutant structures. The best 15 structures of the WT MA and 30 of the T41I/T78I mutant were deposited into the PDB database (PDB ID: 5LMY and 5LDL, respectively). Structure statistics for the re-refined WT structure and the T41I/T78I and Y28F/Y67F MA mutants can be found in the Supplementary Data (Supplementary Tables 1–3). The part of RDCs that was not used for structure calculation was used for structure validation (Supplementary Figs. 1 and 2). RDCs obtained for the Y28F/Y67F MA mutant were not used in structure determination but were used solely for structure validation (Supplementary Fig. 3).

The global fold of the T41I/T78I MA mutant was very similar to the WT MA. However, a few differences between these two structures were found (Fig. 1). Helix IV in the mutant structure was broken into two parts. This was likely caused by the contacts of isoleucine introduced by the T78I mutation with the residues K27 and L32 from the second helix (based on found NOE contacts). The mutation T41I changed the curvature of the loop between the second and the third helices. This resulted in the reorientation of the second helix by approximately 90 degrees around the helical axis. The N-terminal part of the second helix is also shifted closer to the myristoyl.

Relatively more significant structural changes were induced by the Y28F/Y67F MA mutations (Fig. 2). The first helix was broken into two shorter helices oriented