Biochimica et Biophysica Acta 1859 (2017) 1335–1349

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Membrane localization and dynamics of geranylgeranylated Rab5 hypervariable region

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ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 18 April 2017
Accepted 23 April 2017
Available online 25 April 2017

Keywords:
Small GTPase
Peripheral membrane protein
Molecular dynamics
Hypervariable region
Lipid enrichment
Lipid-protein interactions

ABSTRACT

The small GTPase Rab5 is a key regulator of endosomal trafficking processes and a marker for the early endosome. The C-terminal hypervariable region (HVR) of Rab5 is post-translationally modified at residues Cys^{212} and Cys^{213} to accommodate two geranylgeranyl anchors (C20 carbon chain length) in order to associate Rab5 with the membrane. The structural role of the HVR regarding protein-early endosome membrane recruitment is not resolved due to its high degree of flexibility and lack of crystallographic information. Here, full-atomistic and coarse-grained molecular dynamics simulations of the truncated Rab5 $HVR^{206–215}$ in three model membranes of increasing complexity (pure phospholipid bilayer, ternary membrane with cholesterol, six-component early endosome) were performed. Specific electrostatic interactions between the $HVR^{206–215}$ Arg^{209} residue and the phosphate group of the inositol ring of PI(3)P were detected. This shows that PI(3)P acts as a first contact site of protein recruitment to the early endosome. The free energy change of $HVR^{206–215}$ extraction from the bilayer was largest for the physiological negatively charged membrane. 5 μs coarse-grained simulations revealed an active recruitment of PI(3)P to the $HVR^{206–215}$ supporting the formation of Rab5- and PI(3)P enriched signaling platforms.

1. Introduction

Rab GTPases are key regulators of vesicular transport between different intracellular compartments in eukaryotic cells. As peripheral membrane proteins they are post-translationally modified by fatty acid chains which anchor them to the membrane. This lipid anchor is covalently attached within a C-terminal hypervariable region (HVR) which has a disordered structure and is thus highly flexible. The role of the HVR is controversially discussed in literature [1,2] and it remains still unclear if Rab targeting to specific cellular membranes is HVR-dependent. The HVR is the linker between the catalytic GTPase domain (G domain) and the membrane anchor; however, its concrete function varies between different members of the Rab family. In some cases, e.g. Rab7 and Rab35, the HVR is essential for specific Rab targeting [3]. Hydrophobic lipid anchors are distinguished according to their structure (branched or unbranched) and their chain length. Common lipid anchors are branched isoprenyl groups like geranylgeranyl or farnesyl chains, saturated fatty acids like palmitoyl chains, sterol groups or glycophosphatidylinositol (GPI) anchors. The partitioning of membrane proteins into raft regions, i.e. dynamic lateral substructures rich in sphingolipids and cholesterol [4,5], or non-raft regions appears to depend on the type of lipid modification. Saturated lipid moieties, GPI as well as sterol anchors are known to target proteins into raft-like domains. On the other hand, short unsaturated or branched lipid modifications counteract a partitioning into highly ordered raft domains [6]. However, it is still under debate to what extent lipid anchors affect protein sorting and membrane organization and which other factors may be involved [7–9]. Observations suggest that apart from pure liquid ordered (Lo)–liquid disordered (Ld) phase separation also the plasma membrane composition with regard to lipid-protein and protein-protein interactions is important for the phase preference of lipid anchored signaling proteins.

In this study we focus on a truncated Rab5 HVR (hereafter, $HVR^{206–215}$) with two geranylgeranyl (GG) chains covalently attached to residues Cys^{212} and Cys^{213} (Fig. 1). Except for membrane binding via its GG anchor little is known about C-terminal Rab5-membrane interactions. Besides mainly polar uncharged amino acids the peptide contains one positively charged arginine residue. Rab5 is associated with the regulation of early endosomal trafficking, vesicle budding, early endosomes fusion, phagocytic transport and micropinocytosis [10]. Previous molecular dynamics (MD) studies of full-length Rab5 revealed two different orientations of the catalytic G domain, resulting in the formation of multiple protein-lipid contacts [11]. A similar behavior with at least two dominant G domain binding modes was observed for full-length H-Ras [12] and K-Ras [13] in atomistic MD simulations. These

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http://dx.doi.org/10.1016/j.bbamem.2017.04.021
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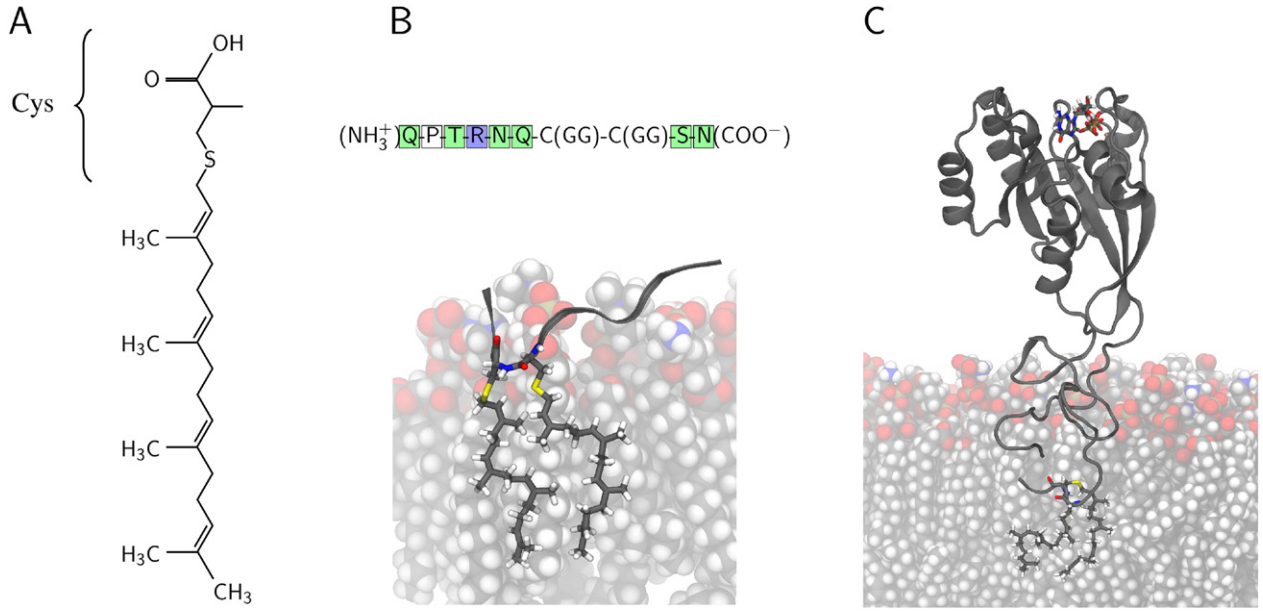

Fig. 1. The geranylgeranyl (GG) chain is covalently attached to a protein cysteine residue (A). Rab5 C-terminal $HVR^{206–215}$ sequence (top) and structure of two GG chains in the membrane (B). MD simulated full-sequence Rab5 protein binding to the membrane (C).

membrane-associated orientational dynamics appear to be a general concept for a wide range of lipidated small GTPases [14]. In order to investigate the interactions between protein and lipids in more detail, model systems representing the membrane-anchored C-terminus of small GTPases, often a short peptide sequence, were analyzed in several studies. Experimental solid-state NMR and Fourier transform infrared spectroscopy as well as MD simulations were used to investigate N-Ras peptide dynamics in a DMPC lipid bilayer [15–19]. Moreover, MD simulations were performed, elucidating different conformational states of the lipidated, positively charged K-Ras C-terminus [20] and point to the role of charged lipids in membrane systems on protein binding [21].

Our MD study investigates the Rab5 $HVR^{206–215}$ bound to membranes of different lipid compositions in a multiscale approach. The highly flexible $HVR^{206–215}$ showed several distinct conformational states. Electrostatic interactions between the charged PI(3)P signaling lipid and the protein arginine amino acid residue were observed and indicate a membrane-protein recruitment site. The potential of mean force of the GG anchor extraction by steered MD and umbrella sampling method showed that these electrostatic interactions with the charged membrane persist after the GG anchor was completely removed from the bilayer. We employed long-term coarse-grained MD simulations to sufficiently sample the lipid diffusion in membranes which revealed a significant accumulation of cholesterol and PI(3)P in close proximity to the peptide.

2. Methods

2.1. Protein structure preparation and parameterization of the lipid anchor

We constructed a $HVR^{206–215}$ model of the C-terminal residues of the Rab5 protein: (NH_3^+)Gln-Pro-Thr-Arg-Asn-Gln-Cys(GG)-Cys(GG)-Ser-Asn(COO^-), with GG presenting the geranylgeranyl chains covalently bound to the cysteine residues Cys^{212} and Cy^{213}. In all-atom simulations the lipid modifications were modeled using parameters derived from the CHARMM36 force field [22,23]. Structural properties of the anchors (bond lengths, bond angles, dihedrals) were validated against optimized structures from quantum-mechanical DFT calculations with TURBOMOLE V6.6 [24]. Geometry optimizations were performed with a split-valence basis set (def2-SVP) [25] using the pure BP86 functional [26,27] and the conductor-like screening model (COSMO) [28] with a dielectric constant of $\varepsilon = 2$ in order to mimic the hydrophobic core region of the surrounding membrane lipids [29].

The protein C-terminus starting structure was obtained from a full-sequence Rab5 structure minimized for 20,000 steps by conjugate gradient and refined in 250 ns MD simulations with the CHARMM36 force field [11].

2.2. Membrane models

Three symmetric model membranes of different lipid compositions were built. For pure palmitoyl-oleoyl-phosphatidylcholine (POPC) the VMD Membrane Plugin [30] was used. The ternary and six-component membranes were built with the CHARMM-GUI Membrane Builder [31,32]. The POPC membrane represents a neutral, zwitterionic model bilayer. The ternary membrane is a combination of lipids often used as a simple model for the plasma membrane featuring cholesterol and sphingolipids which are crucial for ordered raft domains [33]. The composition of the six-component membrane was chosen according to the inner leaflet of the mammalian plasma membrane [34]. The level of phosphatidylinositol 3-phosphate (PI(3)P) is adjusted to that of the early endosome membrane. The lipid compositions and the dimensions of membrane planes are given in Table 1.

Table 1
Composition and lateral dimensions of the three model membrane systems. Abbreviations as follows; POPC: palmitoyl-oleoyl-phosphatidylcholine, CHOL: cholesterol, PSM: palmitoyl-sphingomyelin, POPE: palmitoyl-oleoyl-phosphatidylethanolamine, POPS: palmitoyl-oleoyl-phosphatidylserine, PI(3)P: phosphatidylinositol 3-phosphate.

Membrane system	Component	Number of lipids (ratio)	Lateral (x, y) dimensions/nm
Pure POPC	POPC	2 × 273 (100%)	15.7 × 14.9
Ternary	POPC	2 × 166 (40%)	14.8 × 14.4
	CHOL	2 × 166 (40%)	
	PSM	2 × 83 (20%)	
Six-component	POPC	2 × 90 (17.8%)	16.9 × 16.6
	CHOL	2 × 150 (29.7%)	
	PSM	2 × 50 (9.9%)	
	POPE	2 × 135 (26.7%)	
	POPS	2 × 55 (10.9%)	
	PI(3)P	2 × 25 (5.0%)	