Ras proteins are membrane-associated, small guanine nucleotide-binding GTPases that control cell survival and proliferation. They consist of highly homologous catalytic domains and flexible C-terminal hypervariable regions (HVRs) that differ significantly across Ras isoforms, H-Ras, N-Ras, K-Ras4A and K-Ras4B, where K-Ras4B is among the frequently mutated oncogenes in human tumors. Recent NMR experiments have discovered that the HVR of GDP-bound K-Ras4B extensively interacts with the catalytic domain. However, the HVR weakly interacts with the catalytic domain when the protein is in the GTP-bound state. Molecular dynamics (MD) simulations confirmed tight interaction of HVR with catalytic domain in the GDP-bound state, but not GTP-bound K-Ras4B, suggesting that in the GDP-bound state, an HVR domain could adopt a β-strand conformation, extending the β-sheet in the active site of the catalytic domain. Here we modeled K-Ras4B membrane interaction and dimerization. Membrane binding of K-Ras4B through the anchoring of the positively charged HVR is critical to its function as an oncogene and initiates signaling events. Recent studies showed that post-translationally modified HVR peptide spontaneously inserts the farnesyl group into the zwitterionic (DOPC) and anionic (DOPC:DOPS=4:1) bilayers in the liquid phase, but not into the DPPC bilayer in the gel phase. Further, spontaneous membrane insertion of the farnesyl group in full-length K-Ras4B strongly depends on the nucleotide type. The HVR of K-Ras4B-GTP preferentially interacts with lipids through the farnesyl insertion, while the HVR of K-Ras4B-GDP rather binds the catalytic domain and inserts less frequently the farnesyl into the lipid bilayer. Remarkably, K-Ras4B-GTP, but not GDP-bound, is able to form stable homodimers with different dimer interfaces, suggesting that the nucleotide-dependent dimerization with various dimer interfaces can resolve nanoclustering and cluster reorganization. Does the formation of homo-oligomers interfere with the assembly of the hetero-oligomer? We studied the insertion of the putative POPD transmembrane segment into the membrane we noticed that the location of the segment differed in the presence and in the absence of POPD. Formation of a heterocomplex positioned the POPD segment into a more hydrophobic environment. We reasoned that this conformational change could be employed to determine if the conformation adopted by POPD in homo-oligomers is affected by the subsequent addition of POPB. If reversible, formation of hetero-oligomers will change the location of the transmembrane segment to a more hydrophobic environment. An analytical framework that uses energy homo transfer to directly probe quantitatively the oligomerization state of membrane-bound proteins engaged in a three-state cooperative partition is presented [1]. It was assumed that monomeric proteins partition into the bilayer surface and reversibly assemble into oligomers with k subunits [2]. A general equation relating the overall steady-state fluorescence anisotropy of the sample to its fractional labeling was derived by considering explicitly that the anisotropy of mixed oligomers containing i-labeled monomers is inversely proportional to the number of labeled subunits per oligomer (Runnels and Scarlata limit). This method was very robust in describing the electrostatic interaction of Alex7 488-fluorescently labeled lysosome (Lz-A488) with phosphatidylserine-containing membranes. The pronounced decrease detected in the fluorescence anisotropy of Lz-A488 always correlated with the system reaching a high membrane surface density of the protein (low L/P molar ratio). The occurrence of energy homo transfer-induced fluorescence depolarization was further confirmed by measuring the anisotropy decays of Lz-A488 under these conditions. A global analysis of the steady-state anisotropy data obtained under a wide range of experimental conditions (variable anionic lipid content of the liposomes, L/P molar ratios and protein fractional labeling) confirmed that membrane-bound Lz-A488 assembled into oligomeric complexes, possibly with a stoichiometry of k=6 ± 1. This study illustrates that even in the presence of a coupled partition/oligomerization equilibria, steady-state anisotropy measurements can be used to monitor the self-assembly of membrane-bound proteins.

References
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