BINDING OF DPPC LIPID TO SEC14 PROTEIN

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Introduction

The major yeast phosphatidylinositol/phosphatidylcholine transfer protein Sec14p is the founding member of a large eukaryotic protein superfamily. Functional analyses indicate the Sec14p integrates phospholipid metabolism with the membrane trafficking activity of yeast Golgi membranes. In this regard, the ability of Sec14p to rapidly exchange the bound phospholipid with the phospholipid monomers that reside in stable membrane bilayer is considered to be important for Sec14p function in cells. The X-ray structure with two detergent molecules bound suggest possible binding site for phosphatidylinositol (PI) lipid. How Sec14p-like proteins bind phospholipids and if there is a difference in binding sites for PI and PC lipids remains unclear.

Experimental

We have investigated conformation of the spin-labeled phosphatidylcholine lipid (n-doxyl-PC) bound to Sec14 protein by measuring accessibility of the spin-label to a neutral paramagnetic water soluble EPR broadening agent. Based on X-ray structure it is proposed that the PC lipid is bound inside the Sec14p lipid binding pocket. Our previous HF EPR experiments aimed to measure local polarity profile along the lipid chain show that lipid is located in environment similar in polarity to internal protein regions. However, carbon positions 5 and 16 along the lipid chain are characterized by high polarity/proticity environment with the nitroxide label existing mostly in the hydrogen-bonded form. Here we have assessed the accessibility of the nitroxide positioned along the lipid chain to solvent molecules by measuring relaxant-induced changes in $T_1$ electronic relaxation rate of the label using pulse saturation recovery experiment.

Results and Discussion

Figure shows the first 4μsec profile of a typical SR EPR signal measured for air-equilibrated samples of Sec14p-bound 5-doxyl-PC with and without 50 mM NiEDDA challenge. Measurements of the paramagnetic relaxant accessibility to the lipid chain show that the positions 5 through 12 are strongly shielded from hydrophilic solute molecules. The distal acyl chain region (position 16) of the bound phospholipid exhibits limited accessibility to hydrophilic paramagnetic broadening agents; ca. 13% of what would be normally observed for solvent-exposed protein residues. Partial exposure of the distal chain to the solvent is in agreement with higher polarity and proticity of this site observed in HF EPR experiments. Observation of the position 5 being completely shielded from the paramagnetic agent suggests that higher polarity and proticity of this site is due to formation of a hydrogen bond with the protein residues rather than with solvent molecules. Overall, observed higher local polarity and partial local accessibility of the distal end to the broadening agent indicates that the tail of the PC lipid could be partially “sticking out” of the lipid binding pocket.

Conclusions

Measurements of the paramagnetic relaxant accessibility to the lipid chain show that the PC lipid is strongly shielded from hydrophilic solute molecules with exception of the distal tail of the lipid that demonstrates higher local proticity of the environment and a partial accessibility to the broadening agent.

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