

Reference

Utilizing the uniform protocols established by the LIPID MAPS Consortium (www.lipidmaps.org), RAW 264.7 cells were treated with **Kdo₂-Lipid A**, a defined endotoxin that activates macrophages via TLR-4, then the sulfatide amounts and types were analyzed using liquid chromatography, electrospray tandem mass spectrometry. Sulfatides are detected and quantified by multiple reaction monitoring analysis in negative ion mode using an API 4000 quadrupole linear ion trap (varying the collision energy from 80 eV to 130 eV for the internal standard, a C12:0 homolog from Avanti Polar lipids, and the cellular sulfatides with C16:0 to 26:0 ceramide backbones). Although RAW 264.7 cells contain essentially no endogenous sulfatide, there is a large increase beginning approximately 12 h after Kdo₂-Lipid A addition. As best we have been able to ascertain, this is the first finding of induction of sulfatide biosynthesis by macrophage activation, and it will be interesting to determine if the increase in sulfatides has an impact on macrophage function. Quantitative analysis of sulfatides in RAW 264.7 cells treated with Kdo₂-Lipid A. (2007).

Jeremy Allegood, Elaine Wang, M. Cameron Sullards and Alfred H. Merrill, Jr. FASEB Journal. 21:779.12

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Reference

Sec14p promotes the energy-independent transfer of either phosphatidylinositol (PtdIns) or phosphatidylcholine (PtdCho) between lipid bilayers in vitro and represents the major PtdIns/PtdCho transfer protein in the budding yeast Saccharomyces cerevisiae. Herein, we employ multifrequency high field electron paramagnetic resonance (HF EPR) to analyze the electrostatic and hydrogen bonding microenvironments for series of **doxyl-labeled PtdCho** molecules bound by Sec14p in a soluble protein-PtdCho complex. A structurally similar compound, 5-doxyl stearic acid dissolved in a series of solvents, was used for experimental calibration. The experiments yielded two-component rigid limit 130 and 220 GHz EPR spectra with excellent resolution in the gx region. Those components were assigned to hydrogen-bonded and non-hydrogen bonded nitroxide species. Partially resolved 130 GHz EPR spectra from n-doxyl-PtdCho bound to Sec14p were analyzed using this two-component model and allowed quantification of two parameters. Firstly, the fraction of hydrogen-bonded nitroxide species for each n-doxyl-PtdCho was calculated. Secondly, the proticity profile along the phospholipid-binding cavity of Sec14p was characterized. The data suggest the polarity gradient inside Sec14p cavity is a significant contributor to the driving molecular forces for extracting a phospholipid from the bilayer. Finally, the enhanced g-factor resolution of EPR at 130 and 220 GHz provides researchers with a spectroscopic tool to deconvolute two major contributions to the x-component of the nitroxide g-matrix: hydrogen bond formation and local electrostatic effects.

Smirnova, T.Í., T.G. Chadwick, M.A. Voinov, O. Poluektov, J. van Tol, A. Ozarowski, G. Schaaf, M.M. Ryan, and V.A. Bankaitis. (2007). Local Polarity and Hydrogen Bonding inside the Sec14p Phospholipid-binding Cavity: High-Field Multifrequency EPR Studies. *Biophys J* 92(10):3686-95.

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