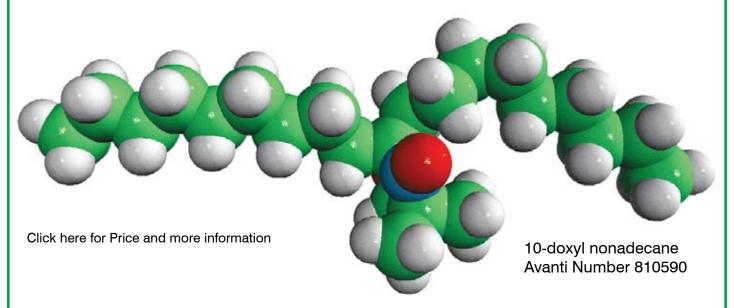
A New Molecule for Trp Quenching Only from Avanti®



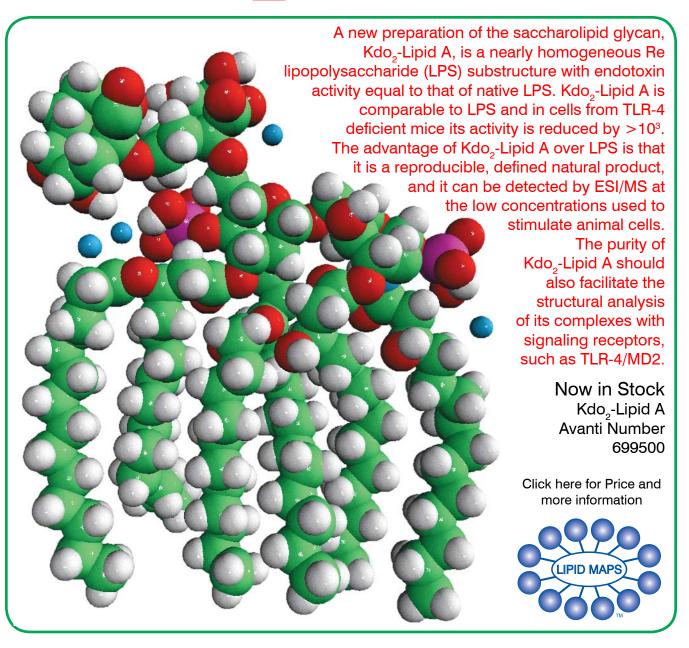
A novel fluorescence method for determining the depth of Trp residues in membrane-inserted polypeptides is introduced. Quenching of Trp by acrylamide and 10-doxylnonadecane (10-DN) was used to measure Trp depth. Transmembrane helices with Trp residues at varying positions (and thus locating at different depths in lipid bilayers) were used to calibrate the method. It was found that acrylamide quenches Trp close to the bilayer surface more strongly than it quenches deeply buried Trp, while 10-DN quenches Trp close to the center of the bilayer more strongly than Trp close to the surface. The ratio of acrylamide quenching to that of 10-DN was found to be nearly linearly dependent on the depth of Trp in a membrane. It was also found that it was possible to detect coexisting shallowly and deeply inserted populations of Trp-containing polypeptides using these quenchers. In the presence of such mixed populations, acrylamide induced large blue shifts in fluorescence emission lambda(max) whereas 10-DN induced large red shifts. In a more homogeneous population quencher-induced shifts were found to be minimal. Dual quencher analysis can be used to distinguish hydrophobic helices with a transmembrane orientation from those located close to the bilayer surface and, when applied to a number of different peptides, revealed novel aspects of hydrophobic helix behavior. Caputo, G.A. and E. London. (2003). Using a novel dual fluorescence quenching assay for measurement of tryptophan depth within lipid bilayers to determine hydrophobic alpha-helix locations within membranes. *Biochemistry* 42:3265-74.

AVANTI'S NITROXIDE SPIN LABELED PC'S

A variety of positions down the hydrophobic chain are labeled with the nitroxide functional group.

This family of compounds allows probing of the membrane to various depths.

AN ENDOTOXIN TO REPLACE LPS KD02-LIPID A



Reference

Raetz, C.R., T.A. Garrett, C.M. Reynolds, W.A. Shaw, J.D. Moore, D.C. Smith Jr, A.A. Ribeiro, R.C. Murphy, R.J. Ulevitch, C. Fearns, D. Reichart, C.K. Glass, C. Benner, S. Subramaniam, R. Harkewicz, R.C. Bowers-Gentry, M.W. Buczynski, J.A. Cooper, R.A. Deems, and E.A. Dennis. (2006). Purification and properties of Escherichia coli Kdo₂-lipid A, a defined endotoxin that activates macrophages via TLR-4. *J Lipid Res* 47:1097-111.

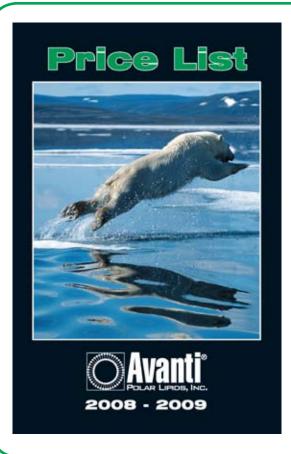
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