Sphingosine-1-phosphate (S1P) is a pro-inflammatory lipid and pro-survival signal generated primarily by phosphorylation of sphingosine via sphingosine kinase 1 (SK1). SK1 is an ~43 kDa enzyme with two domains and an active site within a cleft between the two domains (1). Although the role of SK1 in generating S1P and activating downstream targets is fairly well studied, there has been a paucity of information on how SK1 interacts with lipid membranes/cell membranes where it presumably accesses its substrate, sphingosine. SK1 has been linked to several types of cancers (2) where SK1 was found to be overexpressed (3), and expression levels are linked with patient survival and prognosis as well as chemotherapeutic resistance (4). SK1 also has been shown to have a central role in neurotransmission and endocytosis where SK1 membrane binding and S1P generation were critical for proper endocytic vesicle formation from the plasma membrane (5).

Studies more than a decade ago demonstrated SK1 translocation from the cytosol to the plasma membrane following cellular stimulation with PMA (6) where the membrane fraction containing SK1 has increased levels of phosphorylation of substrate (7). Plasma membrane anionic lipids including phosphatidic acid (PA) and phosphatidylserine (PS) have been shown to stimulate SK1 activity (8) and recruit SK1 to membranes (9). Recent studies investigating a hydrophobic patch exposed on the SK1 structure revealed an important role for hydrophobic residues in SK1 membrane recruitment and loss of function in neurotransmission for hydrophobic mutants of SK1 (5). However, mechanisms by which SK1 is recruited to the membrane surface to interact with anionic lipids necessary for SK1 activity have not been well explored.

In this issue of the Journal of Lipid Research, Pulkoski-Gross et al. (10) examine the ability of SK1 to interact with anionic membranes based on the SK1 structure. Despite the structure of SK1 being solved more than four years ago, there has been little mechanistic information on how SK1 accesses its substrate in the context of membranes. The important new study by Pulkoski-Gross et al. first examined the SK1 structure using computational methods to reveal regions of positive electrostatic potential, as SK1 has been known to interact with anionic membranes. The computational studies revealed a highly positively charged site consisting of Lys27, Lys29, and Arg186 (Fig. 1). Notably, this electrostatic site is on the same interface as a hydrophobic region previously found to regulate SK1 plasma membrane localization (5) and is adjacent to the known binding sites for sphingosine and ATP.

A triple mutation of the cationic site was prepared (K27E/K29E/R186D) to study SK1 lipid interactions in vitro and in cells. A hydrophobic mutation (L194Q) was also prepared so as to study the hydrophobic contributions to binding and activity and also to serve as a control, as mutations to the hydrophobic patch were previously shown to reduce SK1 plasma membrane localization and activity (5). L194Q reduced membrane association of SK1 to PA-containing vesicles whereas the triple cationic site mutant was severely defective in PA vesicle binding, suggesting this site is the main determinant of PA binding.

Experiments with hydrogen-deuterium exchange mass spectrometry (HDX-MS), helped Pulkoski-Gross et al. to cement the foundation of the lipid binding mechanism. In the absence of structural data with the lipid ligand bound, HDX-MS has proved to be a valuable method for dissecting the lipid binding interface of several peripheral proteins (11, 12). HDX-MS is used to measure the rate of exchange of amide hydrogens with solvent in order to study protein structural dynamics. Residues that are found in protein secondary structure will be protected from amide exchange, whereas residues that are involved in lipid binding or conformational change at the membrane interface will see a large decrease in the exchange rate in the presence of lipid. HDX-MS studies with PA-containing vesicles revealed that the SK1 cationic (Lys27, Lys29, and Arg186) and hydrophobic (Leu187, Met189, Leu194) region align to make up a contiguous interfacial binding surface (Fig. 1).

To study SK1 mutant localization and activity in cells, CRISPR/Cas9 was used to knock out SK1 in HCT116 cells so the membrane interaction site mutants could be studied in the absence of competition with endogenous WT
enzyme. In consonance with in vitro studies, both the hydrophobic (L194Q) and cationic triple site mutant greatly reduced the plasma membrane localization and activity compared with WT SK1-expressing cells. A secondary assay in HEK293 cells using green fluorescent protein-tagged constructs and PMA stimulation to induce SK1 translocation also revealed a significant deficit in the hydrophobic and cationic mutant ability to access the plasma membrane.

The physiological relevance of SK1 mutations on SK1-signaling processes was examined by several assays. First, a previous study demonstrated SK1 localized with endophilin-2 in response to membrane perturbations of sphingomyelinase treatment or cholesterol sequestration (5). Pulkoski-Gross et al. found that WT SK1 was able to colocalize with endophilin-2 in response to membrane perturbation, whereas the hydrophobic or cationic mutants disrupted the colocalization with endophilin-2. Further, phosphorylation of ezrin-radixin-moesin (ERM) has been shown to be dependent on SK1 when cells are fed sphingosine. WT-SK1 exhibited significant phosphorylation of ERM in the cell assay, whereas L194Q and the cationic mutant had a minor reduction in ERM phosphorylation. In contrast, mutation of the hydrophobic and cationic site of SK1 together led to a dramatic loss of ERM phosphorylation, suggesting an important role of the contiguous membrane interface in SK1 activation and phosphorylation of ERM. Finally, a cell invasion assay revealed that the HCT116 cells expressing WT SK1 moved more efficiently through the invasion assay compared with the hydrophobic or cationic SK1 mutants. Thus, SK1 plasma membrane binding (through both electrostatic and hydrophobic interactions) is an important and central feature of SK1 activity and downstream signaling.

Although the study by Pulkoski-Gross et al. was the first to provide molecular insight into the residues and interface SK1 requires for PA binding, there are some limitations to the study. For instance, PA can be fully deprotonated at physiological pH, meaning its anionic charge is −2 compared with the −1 charge of abundant plasma membrane lipids such as PS. Thus, the anionic charge-dependent effects of SK1 membrane recruitment will require further investigation. Further, another potentially interesting area of SK1 properties to understand is the role of membrane physical properties, such as curvature, in SK1 membrane recruitment. Binding to anionic lipids PA and PS was significant for SK1 (10), whereas binding to anionic phosphatidylinositol (PI) was limited. Notably, PI has a larger headgroup, whereas PA has a small headgroup and favors negative curvature. Interestingly, binding of SK1 to another lipid with negative intrinsic curvature, phosphatidylethanolamine, was also observed for SK1 and the localization of SK1 to the budding neck of endocytic vesicles, a well-known site of positive (vesicle) and negative (neck region) membrane curvature, has been previously suggested (5).

Thus, mechanistic studies to follow may investigate the ability of SK1 to interact with lipids with different intrinsic curvature properties.

The SK1 Leu194 residue, which was shown to be an important part of membrane binding activity of SK1 in the study by Pulkoski-Gross et al. (10) as well as a previous study (5), is part of an α-helix with several exposed hydrophobic residues (Leu187, Met189, and Leu198) (Fig. 1). It seems likely this interface may protrude into the hydrophobic region of the plasma membrane or interact at the neck of budding endocytic vesicles where there may be some packing defects in the membrane. These hydrophobic interactions may contribute to the necessary site-specific localization of SK1 at the plasma membrane complementing the interaction of Lys27, Lys29, and Arg186 with PA. In closing, the new study by Pulkoski-Gross et al. is an important one as its brings new biophysical methods to understanding the relationship between SK1 structure and function at the membrane interface. Moreover, this study should aid in better understanding of how to therapeutically target this pharmacologically important enzyme.
REFERENCES


