Signaling at the membrane interface by the DGK/SK enzyme family

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Abstract The sphingosine (SK) and diacylglycerol (DGK) kinases have become the subject of considerable focus recently due to their involvement as signaling enzymes in a variety of important biological processes. These lipid signaling kinases are closely related by sequence as well as functional properties. These enzymes are soluble, yet their substrates are hydrophobic. Therefore, they must act at the membrane interface. Second, for both of these enzyme families, their substrates (diacylglycerol for DGKs, sphingosine for SKs) as well as their products (phosphatidic acid for DGK, sphingosine-1-phosphate for SK) have signaling function. To understand how the signaling processes emanating from these kinases are regulated it is critical to understand the fundamental mechanisms that control their enzymatic activity. This is particularly true for the rational design of small molecules that would be useful as therapeutic compounds. Here we summarize enzymological properties of the diacylglycerol and SKs. Further, because the three-dimensional structure of the eukaryotic members of this family has yet to be determined, we discuss what can be gleaned from the recently reported structures of related prokaryotic members of this enzyme family.—Raben, D. M., and B. W. Wattenberg. Signaling at the membrane interface by the DGK/SK enzyme family. J. Lipid Res. 2009. 50: S35–S39.

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Two important and related families of lipid kinases are the sphingosine (SK) and diacylglycerol (DGK) kinases. Both of their substrates (diacylglycerol and sphingosine) and products [phosphatidic acid (PA) and sphingosine-1-phosphate] have important signaling roles. Given the critical function of these lipids in signaling events, it’s not surprising that there is increasing interest in understanding the mechanisms involved in regulating their activities.

This is particularly relevant as these enzymes are being pursued as drug targets in cancer, inflammatory disease, and transplantation. We recently reviewed the current state of our understanding regarding the basal and induced subcellular localization of these enzymes (1). In this review, therefore, we will address their basic enzymology and how it is affected by interaction with other proteins, lipids, and membrane surfaces. Substrate specificity, clearly an important part of enzymology of these enzymes, has been recently reviewed and so will not be addressed here (1).

SKS AND DGKS: SOLUBLE ENZYMES ACTING AT THE MEMBRANE INTERFACE

DGKs and SKs act at a membrane interface and involve lipophilic substrates and products. How do these find their way to and interact with membranes? How do these enzymes recognize and extract their substrates from the membrane milieu? Here, we first summarize the basic enzymology of these enzymes and then discuss how these enzymes interact with the membrane bilayer. In the final section, we discuss the structure of two prokaryotic enzymes, DgkB from Staphylococcus aureus and YegS from Salmonella, with significant homology to the eukaryotic DGKs and SKs.

Activity at the membrane surface: scooting and hopping

Soluble enzymes that utilize membrane-bound lipid substrates are referred to as interfacial enzymes. These enzymes interact with an interface and with a substrate within the interface. Two major approaches suggested for the analysis of these enzymes are “surface dilution kinetics” (2) and “interfacial kinetic analysis” (3). Both approaches consider the dependence of the enzyme on the bulk as well
as surface concentrations of substrates and products. In general, these analyses characterize these enzymes as being either “scooters” or “hoppers”. Scooters have a long residence time at the interface. Hoppers bind relatively transiently to a membrane interface and are less precessive. Enzymes may show intermediate characteristics between these extremes. Whereas initial studies of the interfacial kinetics of at least one mammalian DGK have been reported (4), little is known about this important aspect of SKs.

SKs

What is known about SK interfacial or surface dilution kinetics is derived mainly from a study with partially purified enzyme (5). These data indicate that SK acts as a “scooter”.

DGKs

Of the 10 eukaryotic DGKs (1), all except DGK-ε are largely soluble. DGK-γ is soluble but principally associated with the cytoskeleton in Purkinje cells (6). For the most part, strict kinetic analyses on purified DGKs have been lacking. However, one isoform, DGK-θ, has been examined and appears to be a “quasi-scooter” indicated by its sensitivity to both the bulk (total) and surface concentration of substrate (4). This behavior suggests the enzyme binds to membranes with an interface residence time that is intermediate between a scooter and a hopper.

ACTIVATION BY LIPIDS

SK

Acidic phospholipids enhance the activity of SKs in vitro. Phosphatidylserine (PS) and PA appear to be the most relevant lipids for SK 1, although phosphatidylinositol may also stimulate activity (7). SK 2 is also activated by PS (8). One elegant study directly tested the role of PA to activate SK in a physiological setting using manipulation of phospholipase D (PLD) to generate phosphatidic acid in cells (9). These workers demonstrated that ectopic expression of PLD leads to SK translocation to perinuclear sites where PLD is concentrated. Further, blocking PA production with butanol prevented agonist-dependent Fc receptor activation induced SK membrane translocation (9). This involvement of PA stands in contrast to direct biophysical measurements of SK-1 binding to lipid surfaces (10). Those studies measure a strong effect of PS on SK membrane binding, with little or no effect of PA. Additionally, PS was a much more potent activator of enzymatic activity. These studies demonstrated that serine 225 phosphorylation was required for the PS-dependent lipid association of SK 1.

Several questions remain concerning the lipid activation of SK. Do acidic phospholipids increase enzyme activity primarily by increasing the residence time of SK at the membrane surface, and therefore increase its precessivity? Alternatively, do these lipids result in a change in the intrinsic activity of the enzyme? Detailed studies of the interfacial or surface dilution kinetics of SK will resolve this question. The question of which lipids are relevant in vivo, and under what circumstances, is a crucial issue. Whereas levels of PA may be regulated by agonist-dependent activation of PLD, levels of PS are relatively stable. Regulation by PA would therefore be directly controlled by changes in lipid levels. Regulation by PS could result from either posttranslational modifications of SK (such as phosphorylation) that alter its interaction with the lipid, or accessory factors that direct SK to the membrane. One exception to this may be during the later stages of apoptosis, where PS becomes depleted on the cytoplasmic leaflet of the plasma membrane. It would be predicted that this would reduce association of SK with the plasma membrane, and, considering the antiapoptotic effect of SK-1, would serve to accelerate the apoptotic process.

DGKs

Similar to the SKs, a number of membrane lipids have been shown to modulate DGK activity although their physiological relevance is uncertain. Among the identified lipid modulators of DGKs, PS is one of the best characterized. This lipid activates Type I DGKs, such as DGK-α (11), as well as Type IV DGKs (12) and the lone member of Type V, DGK-θ (4). On the other hand, this lipid inhibits DGK-ε and Type II DGKS. Other lipids have also been shown to modulate certain DGKs. Sphingosine activates DGK-α, whereas DGK-ε is inhibited by phosphoinositides. In this regard, it is interesting that PI(3,4,5)-trisphosphate activates DGK-ε, whereas DGK-β is not affected by this lipid but is activated by PI(4,5)-bisphosphate (13). Interestingly, the product of DGK activity, PA, has been shown to activate DGK-θ as well as DGKA from Dictyostelium. As above, the mechanism by which these lipids modulate DGK activity is not clear.

There is speculation that DGKs may be responsive to diacylglycerol (DAG). This notion derives from the observation that all DGKs contain at least one cysteine-rich domain (CRD), referred to as C1 domains, as these domains in protein kinase C bind PMA. Consistent with this, treatment of cells with PMA induced a translocation of DGK-γ to the plasma membrane (14). Further, C1A domains of DGK-γ and DGK-β bind phorbol ester (14, 15). Complicating these results, DGK-α does not translocate to the plasma membrane in response to PMA and intact DGK-γ and DGK-β failed to bind this molecule. Additionally, C1 domains of other DGKs lack the high sequence homology to the PKC C1 domain observed in the C1A domains of DGK-γ and DGK-β. Nonetheless, DAG levels may play a role in modulating some DGK isoforms. For example, DGK-θ is responsive to total DAG levels following mitogen (thrombin)-stimulation of quiescent fibroblasts. Whereas the activity of DGK-θ presented in lysates isolated from quiescent fibroblasts is unresponsive to bulk (total) DAG levels, mitogen stimulation increased the apparent $K_m$ of DGK-θ for DAG at low bulk concentrations of DAG. When the bulk concentration of DAG increased, the apparent $K_m$ decreased to the uninduced level. This, along with the PA-induced activation of DGK-θ mentioned above, may help account for the transient nature of induced DAG production when this isoform is present.
ACTIVATION BY CALCIUM AND PHOSPHORYLATION

SKs

SKs 1 and 2 are both activated by phosphorylation (1). SK 1 is phosphorylated at a single site, serine 225 (human SK 1) (16), apparently by the Map kinase Erk2. The phosphatase PP2A is responsible for removing this activating phosphorylation (17). SK 2 is phosphorylated by Erk1 at two sites, serine 351 and threonine 578 (18). This phosphorylation has two effects, depending on the SK isoform. First, phosphorylation increases the intrinsic activities of both SK 1 and 2. This increase is approximately 14-fold for SK1 and is clearly physiologically important (19). Second, this phosphorylation is required for agonist-dependent membrane translocation of SK 1, and (see above) for binding of its localization but negatively regulates its activity (26–28).

DGKs

In addition to lipids, calcium is a modulator of the Type I DGKs. There is evidence to implicate the binding of calcium to EF hand motifs leading to the increase in DGK-α activity in vitro (21). The data suggest that the EF hands represent autoinhibitory regions. In this model, calcium activates DGK-α by ablating this autoinhibition.

Three mammalian DGKs, DGK-α, DGK-δ, and DGK-θ, have been shown to be modulated by phosphorylation. DGK-α may be phosphorylated by a number of protein kinases, including some PKC isoforms (22) and Src (23). DGK-δ is phosphorylated on serine residues within its PH domain that appears to be mediated by conventional PKCs and results in the reduction of its translocation to the plasma membrane (24). Finally, phosphorylation of DGK-θ is correlated with its membrane association. DGK-θ complexes with PKC-ε and PKC-η and can be phosphorylated by these PKCs in vitro (25). Compelling evidence indicates that PKC-ε is responsible for the phosphorylation of this DGK isoform in cells. In contrast, phosphorylation DGK-γ also regulates its localization but negatively regulates its activity (26–28).

STRUCTURAL PREDICTIONS FROM PROKARYOTIC HOMOLOGS

Despite abundant interest, no reports of successful crystallization of either an SK or a eukaryotic DGK have emerged. There has been some success in using mutagenesis to identify critical residues that affect interaction either with substrates or membrane surfaces (discussed below). However, this information is incomplete without a three-dimensional structure to integrate the mutational information. Advances have been made, by analyzing structures of prokaryotic proteins that are related, albeit somewhat distantly, to their eukaryotic cousins. Of particular interest is the recent report of the crystal structure of a soluble, prokaryotic DGK (DgkB) (29). This enzyme, as well as eukaryotic diacylglycerol, sphingosine, and ceramide kinases, show weak but significant sequence homology to a larger group of kinases that include phosphofructokinases and polyphosphate/ATP NAD kinasesDGK (30). One defining characteristic of this family is a motif (φφφG/SGDG) that defines an element in the ATP-binding site. There are, however, other areas of homology as well. It is interesting to note, however, that one recently identified DGK in yeast (DGK1) uses CTP in lieu of ATP and lacks a canonical ATP binding site (31). A number of structures of phosphofructokinases have been solved, and these exhibit a two-domain structure, meeting at a hinge that defines a deep cleft. The structures of two family members are of particular note. One is the DgkB structure mentioned above. The second is that of a Salmonella protein of undetermined function, YegS (32). Comparison of these structures has yielded insight into potential substrate-binding regions, sites of regulatory lipid(s) binding, sites of membrane binding, and conformational changes that could modulate activity.

The DgkB structure, like the structures mentioned above, consists of two domains, termed Domains 1 and 2 (Fig. 1). Domain 1 is derived primarily, but not exclusively, from N-terminal sequences and contains the highly conserved ATP binding residues. Domain 2 contains a highly conserved glutamate (Glu273, Fig. 1). This glutamate is proposed to extract the hydrogen from the lipid substrate hydroxyl that is attacked by the γ phosphate of ATP. The site of lipid binding has not been directly determined. Comparison of the DgkB and YegS structures suggests the lipid substrate binding domain consists at least in part of an unstructured loop (between helix 6 and Beta strand 8, Fig. 1) that faces the cleft between the two domains. Binding of substrate along this loop could orient the lipid toward the γ phosphate of bound ATP and position it near the putative catalytic residue, Glu273. Additional evidence for this sequence participating in substrate recognition comes from introduction of a mutation in this region of mouse SK (33). This mutation dramatically increases the Km for sphingosine without affecting the Km for ATP.

More speculative are predictions of the regions involved in binding activating lipids and, perhaps separately, binding to the membrane surface. The YegS structure contains a hydrophobic region on the surface of Domain 1 that faces away from the substrate binding cleft (32). This region could represent a site for binding of lipids responsible for allosteric activation. Miller and colleagues (29), based on the dimeric DgkB structure in their crystals, suggested that the membrane binding region spans domains 1 and 2, next to the ATP-binding site (toward the bottom of the structure as presented in Fig. 1), to orient the cleft between the two for extraction of the lipid substrate into the substrate binding pocket. Clearly, more detailed mutagenesis studies of SK and DGK based on homology modeling will be required to substantiate what appears to be a highly conserved structure. The two-domain structure in this family of enzymes suggests an interesting mode of regulation. The ATP-binding site and putative lipid substrate binding site reside on separate lobes of the enzyme. As suggested by Miller and colleagues (29), movement of the two domains around the hinge region could well regulate catalysis by altering the orientation and spacing in the cat-
Fig. 1. Crystal structure of DgkB from *Staphylococcus aureus* (data from PDB 2qv7, http://www.rcsb.org/pdb/results/results.do?outformat=). A: Space filling model of the structure. Acidic regions are shown in red and basic regions in blue. The site of ADP binding is noted. The two hinged lobes of the structure are denoted as Domain 1 and Domain 2. B: Ribbon model of the structure. Shown in stick representation is the highly conserved Gly-Gly-Aps-Gly ATP-binding motif characteristic of this family of kinases, the loop between β strand 8 and α helix 6 proposed to be involved in substrate binding, the proposed catalytic residue Glu273, as well as ADP. C: Stick representation of the sequences involved in ATP binding and proposed to be involved in catalysis and substrate binding.
alytic site. The relatively modest changes in catalytic activity in this family of kinases may well be a consequence of movements around this hinge induced by post translational modifications, such as phosphorylation or binding to membranes, lipids, or auxiliary proteins.

**SUMMARY**

Interest in the SK and DGK family of lipid kinases continues to increase as we learn more about their potential physiological roles. Whereas much has been learned, understanding of the molecular mechanisms regulating the membrane association and catalytic activity of these enzymes is a major challenge. Protein-protein, protein-lipid, and post-translational modifications all appear to play some role in these processes. As our understanding of the molecular details of their translocation and catalytic activity grows, new insights into the structure-function of these enzymes and their potential utility as therapeutic targets will emerge.

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**REFERENCES**


