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Diacylglycerol Kinases: Relationship to Other Lipid kinases

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Abstract

Lipid kinases regulate a wide variety of cellular functions and have emerged as one of the most promising targets for drug design. Diacylglycerol kinases (DGKs) are a family of enzymes that catalyze the ATP-dependent phosphorylation of diacylglycerol (DAG) to phosphatidic acid (PtdOH). Despite the critical role in lipid biosynthesis, both DAG and PtdOH have been shown as bioactive lipids mediating a number of signaling pathways. Although there is increasing recognition of their role in signaling systems, our understanding of the key enzymes which regulate the balance of these two lipid messages remain limited. Solved structures provide a wealth of information for understanding the function and regulation of these enzymes. Solving the structures of mammalian DGKs by traditional NMR and X-ray crystallography approaches have been challenging and so far, there are still no three-dimensional structures of these DGKs. Despite this, some insights may be gained by examining the similarities and differences between prokaryotic DGKs and other mammalian lipid kinases. This review focuses on summarizing and comparing the structure of prokaryotic and mammalian DGKs as well as two other lipid kinases: sphingosine kinase and phosphatidylinositol-3-kinase. How these known lipid kinases structures relate to mammalian DGKs will also be discussed.

1. Introduction

Diacylglycerol kinases (DGKs) are transferases that play essential roles in the physiology of a number of cell types (Baldanzi, 2014; Merida et al., 2017; Shulga et al., 2011a; Tu-Sekine and Raben, 2011). These enzymes catalyze the phosphorylation of diacylglycerol to generate phosphatidic acid and use ATP as the phosphate donor with the exception of the yeast DGK which uses CTP (Han et al., 2008). In contrast to our understanding of the structure and catalytic mechanism of other lipid and protein kinases, understanding of DGKs is limited.

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Conflict of interest

There are no conflicts of interest associated with the information in this review.

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This is particularly true for the mammalian DGKs as much more is known about prokaryotic DGKs.

Interest in DGKs increased as it became clear that not only are they important for lipid homeostasis, they serve to modulate the relative levels of diacylglycerol (DAG) and phosphatidic acid (PtdOH) that play critical roles in a variety of signaling pathways (Eichmann and Lass, 2015; Liu et al., 2013) including neurotransmission (Raben and Barber, 2017; Tu-Sekine et al., 2015; Tu-Sekine and Raben, 2011). This highlights the need to understand the structure, regulation and catalytic mechanism of these enzymes. Indeed, lacking of the molecular and structural insight into DGKs has been a huge barrier for designing highly specific inhibitors for the DGKs to probe further roles and potential therapeutic strategies where possible.

In this review, we discuss what is known about prokaryotic and mammalian DGKs as well as two other lipid kinases; sphingosine kinase and phosphatidylinositol-3-kinase. We further discuss how our currently understanding may provide insights into the catalytic mechanism and regulation of mammalian DGKs.

2. Overall structural features of lipid kinases

Despite the differences in lipid substrates, an overall structure of lipid kinases must contain: a region/residue(s) for membrane association/stabilization, a domain to bind and orient the phosphor donor (ATP/CTP), and a domain for the binding and orientation of the phosphoryl acceptor (lipid substrate). These domains are oriented in a manner to permit the catalytic reaction (Cheek et al., 2002; Fabbro et al., 2015). While we have gained valuable insights into the nucleotide binding domains, the structure of the lipid substrate binding is not well understood. Understanding the architecture of these domains and their relationship to each other in each lipid kinase will provide valuable insights into the catalytic mechanism(s) of these enzymes.

3. Prokaryotic DAG kinases

There are two classes of prokaryotic DGKs designated as DGKA and DGKB (Van Horn and Sanders, 2012). Perhaps the most well-understood, and smallest, of these are the DGKAs. There are two types of DGKAs; one found in gram-positive bacteria and the other found in gram-negative bacteria. A hallmark of these DGKs are that they exist as homotrimeric proteins where each monomer contains three transmembrane-spanning regions (Li et al., 2013; Oxenoid et al., 2002). Interestingly, there are some important substrate and mechanistic differences between these two DGKAs. The one found in gram-positive bacteria prefers undecaprenol as a substrate while the gram-negative enzyme prefers diacylglycerol as a substrate although it also uses phosphorylates glycerol, as well as ceramide (Jerga et al., 2007). Mechanistically, it is interesting to note that this DGKA also show hydrolytic (ATPase) activity (Li et al., 2015).

The prokaryotic DgkB from *Staphylococcus aureus* is essential for lipoteichoic acid synthesis (Matsuoka et al., 2011) and its solubility makes it particularly interesting with respect to its relevance to mammalian DGKs. Importantly, this is the first *bona fide* DGK

with a solved structure (Miller et al., 2008). While the primary sequence similarity of DgkB compared to their eukaryotic cousins is low (15%–18%), the catalytic core is conserved (Jerga et al., 2007; Miller et al., 2008). DgkB consists of two domains: D1 and D2 (Figure 1A), where both domains maintain an $\alpha\beta$ fold. The D1 domain contains highly conserved ATP binding residues (Figure 1A and 1C) and the D2 domain has a highly conserved glutamate (Glu 273), which likely serves as a catalytic base. The active site has been found located in the interdomain cleft but the lipid substrate binding site is still unclear. Another interesting point of DgkB structure is although each DgkB monomer is presumably supports catalysis independently, DgkB exist as extended dimers in solution as revealed by sedimentation velocity experiments (Miller et al., 2008). The established dimer interface is formed through the N-terminal domain (NTD-NTD) contacts. This interaction might play an important role by placing three conserved, positively charged surface residues near the negatively charged membrane so as to facilitate the extraction of the lipid substrate (Miller et al., 2008). There are two features of this enzyme that resemble the mammalian DGKs. First, there appears to be a conserved Asp-water-Mg²⁺ complex in the active site, although a second Mg²⁺ is essential to maintain a competent structure. Second, as noted above, the sequence of the catalytic site is conserved between DGKB and mammalian DGKs suggesting similar catalytic chemistries.

4. Mammalian DGKs: Current Knowledge

Despite our understanding of prokaryotic DGKs, our limited understanding of the structure of mammalian DGKs is a major gap in our knowledge. Importantly, major differences exist between prokaryotic and mammalian enzymes often revealing the fact that structural features in the mammalian enzymes have evolved for specific functions, localizations, and regulation. This is reflected in the fact that there are 10 mammalian isoforms not including additional splice variants for some isoforms (Imai et al., 2005; Luo et al., 2004). Our knowledge of the structure of these isoforms is primarily limited to similarities in linear amino acid sequences. While limited, there is probably more structural information regarding DGK- ϵ than any of the other mammalian DGKs. CD spectral analysis of this enzyme indicates it is composed of 29% α -helices and 22% β -strands (Jennings et al., 2017). Additionally, a hydrophobic segment located at N-terminal residues 20–40 appears to be essential for targeting this enzyme to the endoplasmic reticulum (Matsui et al., 2014; Nakano et al., 2016).

With respect to DGK- θ , there are significant differences between this enzyme and DGK- ϵ . DGK- ϵ is inhibited by PtdOH and PtdSer (Shulga et al., 2011a) while DGK- θ is activated by these lipids (Tu-Sekine and Raben, 2012). It is noteworthy that PtdOH competitively inhibits DGK- ϵ suggesting this lipid binds to a site in DGK- θ which is distinct from the binding site in DGK- ϵ . DGK- ϵ is also inhibited by PtdIns(4,5)P₂ while this lipid has not been shown to affect DGK- θ . Further, DGK- ϵ is the smallest of the mammalian DGKs (approximately 64 kD), is predominantly membrane-associated, and is the only mammalian DGK that shows a fairly strong substrate preference (Lung et al., 2009). DGK- θ (approximately 110 kD) is both soluble and membrane-bound and a substrate preference has not been observed. This isoform is also activated by proteins containing a polybasic region but the endogenous activator(s) has not been identified.

It is always tempting to glean as much information as possible from the linear sequences of DGKs which can highlight similar domains that may predict function. It has been known from such an analysis that all mammalian DGKs contain at least two cysteine-rich C1 domains and a putative catalytic domain. There are interesting differences among them, however, that could shed light on their various functions and regulation. The linear sequence of DGK- θ identifies three distinguishing features of this isoform. First, there are three C1 domains in contrast to the usual two found in other mammalian DGKs. Second, this enzyme possesses a sequence homologous to Ras binding domains termed the Ras-association (RA) domain. Finally, there is a proline/glycine rich (PR) domain near its N-terminus. Given the unique nature of these domains with respect to DGKs, their roles have been the subject of a number of hypotheses.

While linear sequences are important, they must be viewed with some caution. One noteworthy example is the C1 domains which have attracted some interest as they are known to bind phorbol esters and DAG (reviewed in (Das and Rahman, 2014). In this, it was not unreasonable to suspect that one or more of the three C1 domains in DGK- θ is likely involved in binding catalytic DAG. The presence of these motifs, however, does not establish catalytic DAG binding. Hurley et al. analyzed 54 C1 domains including six DGKs (α , β , γ , δ , ϵ and ζ) and suggested that of these DGKs, only DGK- β and DGK- γ contained a C1 domain that fit a profile for phorbol ester binding (Hurley et al., 1997). Importantly, the C1 domains of DGK- θ , as well as DGKs δ and η , do not bind DAG (Sakane et al., 1996; Shindo et al., 2003; Shindo et al., 2001). The involvement of these domains in catalysis is further questioned by the observation that the drosophila DGK1 does not contain any C1 domains (Masai et al., 1992), and porcine DGK- α lacking its C1 domains retains catalytic activity with a DAG K_m similar to the wild type enzyme (Sakane et al., 1996). As a result, while the C1 domains may contribute to membrane localization for some DGKs, the catalytic DAG binding site remains unresolved. While these domains are not likely to be involved in binding catalytic DAG, they may bind other lipids or participate in protein-protein interactions as suggested by Shulga et al (Shulga et al., 2011a). In support of this notion, the C1 domain of DGK ζ has been shown to mediate interactions with β -arrestins (Nelson et al., 2007) and Rac1 (Yakubchuk et al., 2005). Finally, it is important to note that there is some evidence suggesting this domain may be involved in membrane association following the activation of some G-protein coupled receptors (van Baal et al., 2005).

The RA and PR domains are also poorly understood. Binding energies derived from *in silico* analyses suggests that the RA domain of DGK- θ does not bind Ras (Kiel et al., 2005). DGK- θ binds to, and is inhibited by the GTP-bound form of another small GTPase, RhoA, but it is not clear that RhoA specifically binds to the RA domain. As PR domain contains a pXPXXP motif (Yu et al 1994), it is also tempting to suggest that they bind SH3-domain containing proteins.

5. Sphingosine Kinase

One of the enzymes closely related to DGKs are the sphingosine kinases (SKs). These enzymes catalyze the conversion of sphingosine (Sph) to sphingosine-1-phosphate (s-1-P). SKs and DGKs are closely related lipid kinase in term of basic enzymology and the

mechanism of regulation and the details are well summarized in (Raben and Wattenberg, 2009; Siow et al., 2015). While there are two SK isoforms, SK1 and SK2, structural information exists for SK1 only. SK1 and its catalytic product, S1P, have been found closely linked to breast cancer and become a new target for breast cancer treatment (Geffken and Spiegel, 2018). Similar to DgkB, SK1 harbors a two-domain architecture with a N-terminal ATP binding domain (NTD) and a C-terminal Sph binding domain (CTD), both maintain an $\alpha\beta$ fold (Adams et al., 2016). The catalytic center is located in between the two domains, with a Asp81 assigned as the likely general base responsible for the deprotonation of Sph (Wang et al., 2013). The ATP binding site is highly homologous with the prokaryotic DgkB as illustrated in Figure 1B and 1D. Unlike DgkB, SK1 has a well-known conserved lipid substrate binding site in the CTD that makes extensive surface contact with the Sph substrate and the access of Sph to the lipid binding site is likely mediated by the opening and closure of a lipid binding loop (Adams et al., 2016). In addition, similar to DgkB, SK1 has a putative dimerization interface through NTD-NTD interaction. This dimerization is thought to be functional important to extract the lipid substrate from membrane because it leads to the exposure of both lipid binding loop on SK1 to the membranes and at the same time, direct a positively charged concave surface in the dimer interface to the negatively charged membrane (Adams et al., 2016). Recently, Pulkoski-Gross et al demonstrated that SK1 contains a positively charged and hydrophobic motifs together mediates the interaction of this enzyme with membranes (Pulkoski-Gross et al., 2018).

6. PIK superfamily: model of PI3K α (p110 α /p85 α complex)

Phosphoinositide lipid kinases (PIKs) are enzymes that catalyze the generation of specific phosphorylated variants of phosphatidylinositols (PtdIns) (Fruman et al., 1998). This superfamily of lipid kinase can be further divided into three major families according to the position of the inositol ring that get phosphorylated: the PtdIns 3-kinases (PI3Ks), PtdIns 4-kinases (PI4Ks), and PtdIns-P (PtdInsP) kinases (PIP4K and PIP5K) (Brown and Auger, 2011). Among all the PIKs, the structure of PI3K have been studied intensively likely because it's central role in tumor biology (Braccini et al., 2015; Costa et al., 2018; Follo et al., 2015; Janku et al., 2018; Liu et al., 2018). PI3K are a family of lipid kinases capable of catalyzing the phosphorylation of the 3' hydroxyl group of the inositol ring. Based on primary structure and regulatory domain similarity, PI3Ks are further grouped into three classes (Leevers et al., 1999). Here we just focus on PI3K α , one member from class I, as an example to explore how the molecular structure of PIK is correlated to its specific catalytic mechanism.

Unlike DGKB and SK1, which are homodimer and monomer in solution, respectively, PI3K α is a heterodimer composed of an 85 kD regulatory subunit (p85 α) and a 110 kD catalytic subunit (p110 α). Each subunit contains 5 domains respectively as illustrated in Figure 2A. p110 α contains an adaptor-binding domain (ABD), a Ras-binding domain (RBD), a C2 domain, a helical domain, and a kinase domain (Amzel et al., 2008; Gabelli et al., 2010; Huang et al., 2007). p85 is composed of an SH3 domain, a GAP domain, an N-terminal SH2 (nSH2) domain, an inter-SH2 domain (iSH2), and a C-terminal SH2 domain (cSH2) (Gabelli et al., 2010; Huang et al., 2007). As the full-length structure of p110 α /p85 α complex is not available, a truncated enzymatic active complex p110 α /iSH2, which contains

full length of p110 α and the iSH2 domain of p85 α was used to illustrate the organization of PI3K α (Huang et al., 2007).

The overall shape of p110 α /iSH2 is dramatically different from DGKB or SK1, instead of forming a homodimer, p110 α /iSH2 heterodimer has a triangular shape with iSH2 laying on the top and ABD, RBD, helical and kinase domain of p110 α sitting in the bottom (Figure 2B)(Gabelli et al., 2010; Huang et al., 2007). The C2 domain of p110 α is in close proximity to iSH2 and both of them are thought to directly contact the membrane (Gabelli et al., 2010; Huang et al., 2007). While the N terminus of p110 α is mainly involved in interacting with the regulatory subunit, the C-terminal catalytic domain of p110 α maintains a conserved two lobes structures of $\alpha\beta$ fold common to general kinase. The ATP binding site locates in the cleft of the two lobes as illustrated in Figure 2B (Gabelli et al., 2010).

With respect to its catalytic mechanism, PI3Ks have an interesting history. While this enzyme is known for its lipid kinase activity, it also has protein kinase activity (Dhand et al., 1994; Foukas and Shepherd, 2004). This has led to the recognition that it has dual kinase specificity: a lipid kinase activity that catalyzes the ATP-dependent phosphorylation of the 3'-hydroxyl of phosphoinositides and a protein-kinase activity that includes the catalysis of autophosphorylation (Dhand et al., 1994; Foukas and Shepherd, 2004). Recently, critical catalytic residues have been identified as well as a potential catalytic mechanism involving a ternary complex with ATP and PI(4,5)P₂ has been proposed (Maheshwari et al., 2017). The data indicate that Lys776, within the P-loop region, is involved in both lipid and ATP substrate recognition and is critical for autophosphorylation. One interesting observation involved the roles of His936 and His917. While mutation of His917 abolished both lipid and autophosphorylation, mutation of His936 abolished lipid kinase activity only. This is similar to studies of another lipid kinase, a PtdIns-5 kinase (PIKfyve), that also displays protein kinases activity. A double mutation in a putative lipid substrate binding region of this enzyme (K1999E/K2000E) ablates lipid kinase activity but not protein kinase activity (Ikonomov et al., 2002).

7. Lessons from Known Structures and Catalytic Mechanisms

The mammalian SK1 shows interesting structural similarities with the prokaryotic DGKB. Both enzymes have two main domains, with an active site between the two domains, and a flexible hinge likely involved in modulating active and inactive conformations. One reason for this similarity may be related to the fact that DGKB and SK1 are small proteins particularly with respect to the mammalian DGKs. One exception to this is DGK- ϵ but the limited structural studies of this isoform do not permit a good comparison. It seems likely that the other, larger mammalian DGKs will be more closely related to the overall architecture of PIKs. Similar to PI3K, these DGKs have many different isoforms to accommodate its different function/interaction/regulation in various cell types. Given the increased size and guided by linear sequence similarities, it would not be surprising if the N terminus of mammalian DGKs displayed a more complicated organization involved in regulating the catalytic activity of these enzymes.

The catalytic mechanism and molecular mechanisms underlying the regulation of mammalian DGKs is of major interest. This is because this knowledge will not only fill an important gap in our understanding of these enzymes, such information is critical to the generation of specific inhibitors. In terms of catalytic mechanism, it is noteworthy that most kinases utilize a catalytic base that abstracts a proton from the substrate hydroxyl and orients the substrate for in-line attack (associative) on the ATP γ -phosphate (Mildvan, 1997). For DGKB, a glutamate (E273) was proposed to serve this role (Miller et al., 2008). This is similar to the proposed role for glutamate-69 (E69) in DGKA (Li et al., 2015). In sphingosine kinase (SK1), aspartate-61 (D61) is likely the catalytic base for this enzyme (Wang et al., 2013). These data suggest mammalian DGKs will employ an associative mechanism involving a catalytic base. It is noteworthy, however, that there are data to suggest some tyrosine kinases use a dissociative mechanism involving attack on a metaphosphate intermediate (Wang and Cole, 2014). Hopefully, future studies will clarify the catalytic mechanism of the mammalian DGKs.

While a complete review of the molecular mechanisms regulating mammalian DGK activities is beyond the scope of this review, other reviews have covered much of this aspect (Shulga et al., 2011a; Topham and Epand, 2009; Tu-Sekine and Raben, 2011). In general, aside from enzyme expression levels, this regulation may involve one of more of the following: interactions with specific ions, protein-protein or protein-lipid interactions, post-translational modifications, membrane localization/substrate availability. By way of illustration, some isoforms (Class I DGKs) are sensitive to calcium levels, and responses to specific phospholipids varies among the various isoforms (Shulga et al., 2011b; Topham and Prescott, 2002). Additionally, phosphorylation may modulate activities of some DGK isoforms. For example, membrane association of DGK- δ (Imai et al., 2002) and DGK- θ (van et al., 2005), the nuclear localization of DGK- ζ (Topham et al., 1998), and intrinsic activity of DGK- α (Baldanzi et al., 2008) may involve phosphorylations. DGK- δ may also be regulated by oligomerization (Knight et al., 2010). In contrast, our understanding of protein modulators of DGK activities is less clear. DGK- ζ is activated by the hypo-phosphorylated Rb protein (pRb) as well as two related pocket proteins p107 and p130 (Los et al., 2006), while DGK- θ is inhibited by RhoA (Houssa et al., 1999), and is activated by proteins containing polybasic rich regions, termed polybasic activators (PBAs) (Tu-Sekine et al., 2013; Tu-Sekine and Raben, 2012). Importantly, how these mechanisms alter DGK conformations to affect activity remains unresolved. It is tempting to speculate that a hinge region similar to that seen in DGKB may be involved in modulating DGK structures but this appears to be unclear for SK1 (Wang et al., 2013). Clearly, there is a critical need for information regarding the three-dimensional structure of mammalian DGKs which will help elucidate the catalytic mechanisms. Solving these structures by rather conventional approaches, such as x-ray crystallography or NMR, have been hampered largely by the inability to obtain sufficient quantities of highly purified, monodispersed enzymes. The rapid development of single-particle cryo-electron microscopy (cryo-EM) has made it the most compelling technique for solving these challenging protein structures. Indeed, cryo-EM has recently been used to successfully solve the structure of a complex containing a lipid kinase, PtdIns4KIII α , and two regulatory subunits designated TT7 and FAM126 (Lees et al., 2017). Importantly, besides solving large symmetric proteins, cryo-EM has been used to solve the

structure of proteins size around 100 kD. Such small proteins were once thought to be beyond the resolution limit of cryo-EM but recent advances have overcome the original limitations. For example, cryo-EM has been used to determine the structures of isocitrate dehydrogenase (IDH, 93 kD) and lactate dehydrogenase (LDH, 145 kD) at near atomic resolution (Merk et al., 2016). It is hoped that new approaches such as cryo-EM will finally allow us to obtain high resolution structural data of mammalian DGKs.

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Figure 1. DgkB and SK1 overlap structurally. A. DgkB in yellow with the ATP binding site (PDB ID 2QV7). B. SK1 in Marine Blue with the ATP binding site shown with a black box. C. DgkB as an electrostatic surface shown the positive patch close to the ATP binding site (View from the top). D. SK1 as an electrostatic surface shown the positive patch close to the ATP binding site (View from the top).

Figure 2.
Structural model of p110α/iSH2 homodimer. A. Scheme of the p110α/p85α domain organization. B. p110α/iSH2 homodimer with labeled ATP-binding site and ribbon diagram of the kinase domain.