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Molecular properties of diacylglycerol kinase-epsilon in relation to function

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ABSTRACT

The epsilon isoform of mammalian diacylglycerol kinase (DGKε) is an enzyme that associates strongly with membranes and acts on a lipid substrate, diacylglycerol. The protein has one segment that is predicted to be a transmembrane helix, but appears to interconvert between a transmembrane helix and a re-entrant helix. Despite the hydrophobicity of this segment and the fact that the lipid substrate is also hydrophobic, removal of this hydrophobic segment by truncating the protein at the amino terminus has no effect on its enzymatic activity.

The amino acid sequence of the catalytic segment of DGKε is highly homologous to that of a bacterial DGK, DgkB. This has allowed us to predict a conformation of DGKε based on the known crystal structure of DgkB.

An important property of DGKε is that it is specific for diacylglycerol species containing an arachidonoyl group. The region of DGKε that interacts with this group is found within the accessory domain of the protein and not in the active site nor in the hydrophobic amino terminus. The nature of the acyl chain specificity of the enzyme indicates that DGKε is associated with the synthesis of phosphatidylinositol. Defects or deletion of the enzyme give rise to several disease states.

1. Introduction

In mammals there are ten known isoforms of the enzyme diacylglycerol kinase (DGK), as well as gene splice variants. Enzymes of this family all have a homologous active site but differ widely in their molecular mass, mechanisms of activation and biological function (Shulga et al., 2011a). Among these isoforms, only the epsilon isoform of diacylglycerol kinase (DGKε) has a particularly hydrophobic segment that is predicted to be a transmembrane helix. DGKε has several unique features among all the isoforms of DGK (unless otherwise stated, DGK refers to human isoforms, although the sequence is highly conserved among all multicellular eukaryotes). DGKε is the only isoform believed to be permanently attached to a membrane. It is the smallest isoform in terms of molecular mass and it is the only form known to exhibit acyl chain specificity of the lipid substrate, diacylglycerol (DAG). DGKε shows high specificity for 1-stearoyl-2-arachidonoyl glycerol (SAG), the species of DAG with the same acyl chain composition found in the lipid intermediates of the phosphatidylinositol cycle (PI-cycle) (D'Souza and Epand, 2014). All DGK isoforms have two or three C1 domains, but the epsilon isoform is the only one that does not have any other recognizable folding domain.

In the present review we will focus on the nature of the interactions between DGKε and membranes, as well as interactions of this enzyme with its lipid substrate.

2. Membrane binding of DGKε

2.1. Membrane penetration

Fluorescence microscopy and sub-cellular fractionation experiments have demonstrated that DGKε in cells is bound to membranes and is localized at the endoplasmic reticulum (ER) and plasma membrane (PM) (Decaffmeyer et al., 2008; Matsui

Abbreviations: DAG, diacylglycerol; DGK, diacylglycerol kinase; DGKε, the epsilon isoform of diacylglycerol kinase; DOG, 1,2-dioleoyl-glycerol; DOPC, 1,2-dioleoyl-phosphatidylcholine; ECS, electroconvulsive shock; ER, endoplasmic reticulum; HD, Huntington disease; Htt, Huntingtin protein; KO, knockout; LTP, long term potentiation; MEF, mouse embryo fibroblast; PA, phosphatidic acid; PI, phosphatidylinositol; PI-cycle, metabolic cycle to synthesize PI; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_n, all forms of phosphorylated PI; PKC, protein kinase C; PM, plasma membrane; P-motif, phosphate binding motif; SAG, 1-stearoyl-2-arachidonoyl-glycerol; SAPA, 1-stearoyl-2-arachidonoyl-PA; SLG, 1-stearoyl-2-linoleoyl-glycerol; SLPA, 1-stearoyl-2-linoleoyl-PA; NMR, nuclear magnetic resonance; WT, wild-type.

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et al., 2014; Kobayashi et al., 2007). While its localization within these membranes is not unique among DGK isoforms, DGKε is the only isoform that has been predicted and shown to be membrane bound (Decaffmeyer et al., 2008; Glukhov et al., 2007). However, there is one report where DGKε was found on stress fibres in smooth muscle (Nakano et al., 2009). Interestingly, questions remain about the nature of the association of this enzyme with membranes i.e., is it peripherally associated or an integral membrane protein? It should be noted however that there is nothing fundamentally different between integral and peripheral membrane proteins. This is an arbitrary classification based on phenomenology, *i.e.*whether a protein can be extracted from a membrane without disrupting the entire membrane structure. As discussed below, DGKε does not clearly fall into one or the other of these two categories, but is rather a protein whose properties are intermediate between an integral and peripheral membrane protein.

Solubilization studies have shown that high pH or high salt conditions can extract 11% and 14%, respectively, of Flag-DGKε from membranes of COS-7 cells that over-express this protein. Buffers at physiological pH and salt concentration solubilize a negligible amount of Flag-DGKε (Dicu et al., 2007). However, the difference between an integral and a peripheral membrane protein is based on experimental phenomenon and there may not be a clear separation between the two classes. While true integral membrane proteins cannot be extracted from membranes through high pH or salt conditions, the low extraction rates of DGKε suggest that it may be more tightly associated than a typical peripheral membrane protein. DGKε's membrane binding ability is determined by the hydrophobic N-terminal segment comprising of residues 20–42 (residue numbers in this review include the N-terminal Met as residue 1, residue numbering in some of our earlier papers did not include the N-terminal Met, since it is cleaved in the mature protein). This segment of the protein is predicted to form a transmembrane helix with a probability close to 1, according to the program TMHMM Server, v. 2.0 (Fig. 1).

Atomic Force Microscopy studies (in progress) have demonstrated that DGKε forms greater and larger aggregates on a DOPC supported lipid bilayer compared to DGKε Δ40, a truncated form of the protein which has the N-terminal hydrophobic segment (first forty residues) removed. These results are highly suggestive that the N-terminal hydrophobic segment of DGKε is a contributing factor to the observed aggregation. Similar studies have also demonstrated that the number and size of DGKε aggregates are reduced in the presence of a preferred substrate such as SAG. However, this effect is not observed with less preferred substrates such as dilinoleoylglycerol. Similar effects of SAG on aggregation were observed with DGKε Δ40. Therefore, it is hypothesized that the preferred substrate may have a stabilizing effect on a non-aggregated and more active conformation of DGKε.

2.2. N-terminal hydrophobic segment

Deleting the hydrophobic segment of the protein does not alter the acyl chain specificity of DGKε towards arachidonoyl-containing DAG either in the case of DGKε Δ40 (Dicu, Topham, Ottaway, and Epand, 2007) or DGKε Δ58 (Lung et al., 2009). However, this N-terminal segment does play an important role in membrane insertion.

The prediction that the segment of residues (20–42) is transmembrane is supported by experiments based on access to glycosylation in the interior of the endoplasmic reticulum of various constructs containing this N-terminal hydrophobic segment of DGKε (Norholm, Shulga et al., 2011). There is however, some evidence to suggest that this hydrophobic segment forms a re-entrant helix. This is based on the observation that when DGKε, labeled with 3X-FLAG at the amino terminus, is expressed in NIH-3T3 cells, no FLAG epitope is accessible to immunostaining on the exterior of the cell (Decaffmeyer et al., 2008). However, permeabilizing the cell membrane with detergent can access the FLAG epitope. When the Pro residue, near the center of the hydrophobic segment, at residue 33, is mutated to Ala, the FLAG epitope is exposed to the outside of the cell (Decaffmeyer et al., 2008). It is possible that the FLAG tag prevents the peptide from fully entering the membrane, although this would mean that it only prevents the insertion of the wild type protein but not the P33A mutant. There is also a difference in the thickness and lipid composition of the endoplasmic reticulum used for the glycosylation studies (Norholm et al., 2011) and the plasma membrane used for the fluorescence microscopy (Decaffmeyer et al., 2008). In silico analysis suggests that two families of stable conformations exist for this hydrophobic segment and that they are in equilibrium with one other. One of the conformations is a transmembrane helix and the other is a re-entrant helix. These calculations also predict that the mutant P33A would be largely a transmembrane helix as found (Decaffmeyer et al., 2008). It is possible that there is equilibrium between these two conformational forms that can be influenced by relatively small changes in the system such as the nature of the surrounding lipid or interactions with other proteins.

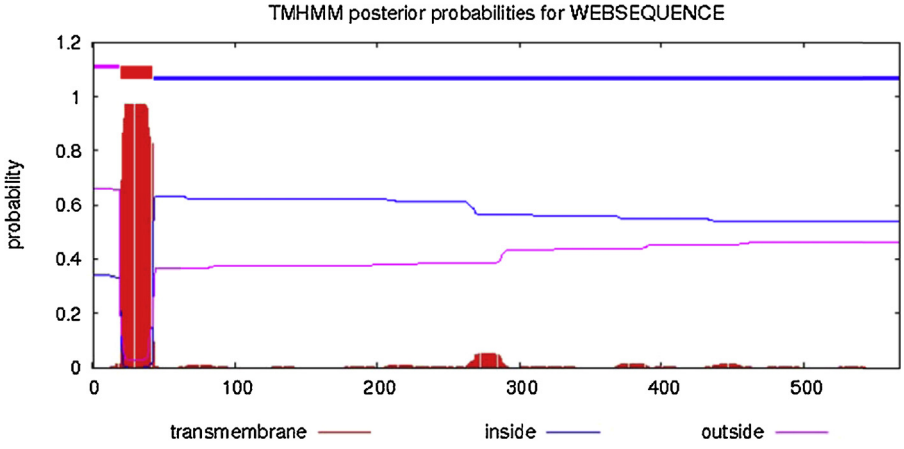

Fig. 1. Using the Web based program for predicting transmembrane helices, TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), a segment near the amino terminus is the only segment predicted to be transmembrane.