Advances in Biological Regulation xxx (2017) 1–8

Contents lists available at ScienceDirect

Advances in Biological Regulation

journal homepage: www.elsevier.com/locate/jbior

Where do substrates of diacylglycerol kinases come from? Diacylglycerol kinases utilize diacylglycerol species supplied from phosphatidylinositol turnover-independent pathways

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ARTICLE INFO

Article history:
Received 1 September 2017
Received in revised form 8 September 2017
Accepted 8 September 2017
Available online xxx

Keywords:
Diacylglycerol kinase
Phosphatidic acid
Monoacylglycerol kinase
Lysophosphatidic acid
Phosphatidylinositol turnover
Phosphatidylcholine-specific phospholipase
Cancer
Diabetes
Parkinson's disease

ABSTRACT

Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DG) to produce phosphatidic acid (PA). Mammalian DGK comprises ten isozymes (α–κ) and regulates a wide variety of physiological and pathological events, such as cancer, type II diabetes, neuronal disorders and immune responses. DG and PA consist of various molecular species that have different acyl chains at the *sn*-1 and *sn*-2 positions, and consequently, mammalian cells contain at least 50 structurally distinct DG/PA species. Because DGK is one of the components of phosphatidylinositol (PI) turnover, the generally accepted dogma is that all DGK isozymes utilize 18:0/20:4-DG derived from PI turnover.

We recently established a specific liquid chromatography-mass spectrometry method to analyze which PA species were generated by DGK isozymes in a cell stimulation-dependent manner. Interestingly, we determined that DGKδ, which is closely related to the pathogenesis of type II diabetes, preferentially utilized 14:0/16:0-, 14:0/16:1-, 16:0/16:0-, 16:0/16:1-, 16:0/18:0- and 16:0/18:1-DG species (*X*:*Y* = the total number of carbon atoms: the total number of double bonds) supplied from the phosphatidylcholine-specific phospholipase C pathway, but not 18:0/20:4-DG, in high glucose-stimulated C2C12 myoblasts. Moreover, DGKα mainly consumed 14:0/16:0-, 16:0/18:1-, 18:0/18:1- and 18:1/18:1-DG species during cell proliferation in AKI melanoma cells. Furthermore, we found that 16:0/16:0-PA was specifically produced by DGKζ in Neuro-2a cells during retinoic acid- and serum starvation-induced neuronal differentiation. These results indicate that DGK isozymes utilize a variety of DG molecular species derived from PI turnover-independent pathways as substrates in different stimuli and cells.

DGK isozymes phosphorylate various DG species to generate various PA species. It was revealed that the modes of activation of conventional and novel protein kinase isoforms by DG molecular species varied considerably. However, PA species-selective binding proteins have not been found to date. Therefore, we next attempted to identify PA species-selective binding proteins from the mouse brain and identified α-synuclein, which has causal links to Parkinson's disease. Intriguingly, we determined that among phospholipids, including several PA species (16:0/16:0-PA, 16:0/18:1-PA, 18:1/18:1-PA, 18:0/18:0-PA and 18:0/20:4-PA); 18:1/18:1-PA was the most strongly bound PA to α-synuclein. Moreover, 18:1/18:1-PA

Abbreviations: DG, diacylglycerol; DGK, diacylglycerol kinase; GRP, guanylnucleotide-releasing protein; KO, knockout; LC, liquid chromatography; LPA, lyso-phosphatidic acid; MG, monoacylglycerol; MGK, monoacylglycerol kinase; MS, mass spectrometry; PA, phosphatidic acid; PC, phosphatidylcholine; PI, phosphatidylinositol; PLC, phospholipase C; PKC, protein kinase C; PS, phosphatidylserine.

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http://dx.doi.org/10.1016/j.jbior.2017.09.003

strongly enhanced secondary structural changes from the random coil form to the α-helix form and generated a multimeric and proteinase K-resistant α-synuclein protein.

In contrast with the dogma described above, our recent studies strongly suggest that PI turnover-derived DG species and also various DG species derived from PI turnover-independent pathways are utilized by DGK isozymes. DG species supplied from distinct pathways may be utilized by DGK isozymes based on different stimuli present in different types of cells, and individual PA molecular species would have specific targets and exert their own physiological functions.

Contents

1. Introduction

Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DG) to produce phosphatidic acid (PA) (Baldanzi, 2014; Goto et al., 2006; Merida et al., 2008; Sakane et al., 2007; Topham and Epand, 2009). To date, ten mammalian DGK isozymes, α, β, γ, δ, ε, ζ, η, θ, ι and κ, have been identified (Fig. 1). Moreover, several alternative splicing products—such as δ1 and δ2 (Sakane et al., 2002); η1–η4 (Murakami et al., 2003, 2016; Shionoya et al., 2015); ζ1 and ζ2 (Ding et al., 1997); and ι1–ι3 (Ito et al., 2004)—have also been found. These isozymes are subdivided into five groups, type I (α, β and γ), II (δ, η and κ), III (ε), IV (ζ and ι) and V (θ), according to structural features (Fig. 1) (Baldanzi, 2014; Goto et al., 2006; Merida et al., 2008; Sakane et al., 2007; Topham and Epand, 2009). Each group is characterized by subtype-specific functional domains, such as EF-hand motifs (type I), pleckstrin homology and sterile α motif domains (type II), ankyrin repeats (type IV) and a ras-associating domain (type V) (Fig. 1).

DGK isozymes regulate a wide variety of physiological and pathological events (Sakane et al., 2007, 2016, 2008). For example, type I DGKα, which is activated in a calcium-dependent manner (Sakane et al., 1990, 1991), is involved in a wide variety of pathophysiological events, such as T-cell anergy induction (Olenchock et al., 2006; Zha et al., 2006), cell motility and invasion (Cutrupi et al., 2000; Rainero et al., 2014), and cancer cell growth/apoptosis (Takeishi et al., 2012; Torres-Ayuso et al., 2014; Yanagisawa et al., 2007). Therefore, a selective and potent inhibitor for DGKα (Liu et al., 2016) can be an ideal anti-cancer drug candidate that attenuates cancer cell proliferation and simultaneously enhances immune responses, including anti-cancer immunity. Knockout (KO) mice of DGKβ exhibited bipolar disorder (mania)-like phenotypes (Kakefuda et al., 2010; Shirai et al., 2010). DGKγ regulated lamellipodium formation (Tsushima et al., 2004), antigen-induced mast cell degranulation (Sakuma et al., 2014) and insulin secretion (Kurohane Kaneko et al., 2013). DGKδ positively regulated epidermal growth factor receptor signaling (Crotty et al., 2006), and DGKδ deficiency also caused hyperglycemia-induced peripheral insulin resistance and thereby exacerbated the severity of type II diabetes (Chibalin et al., 2008). In addition, brain-specific conditional DGKδ-KO mice showed obsessive compulsive disorder-like behaviors (Usuki et al., 2016). DGKη acts as a critical regulator of B-Raf/C-Raf-dependent cell proliferation (Yasuda et al., 2009), and DGKη-deficient mice demonstrated bipolar disorder (mania)-like phenotypes (Isozaki et al., 2016). DGKκ is implicated in fragile X syndrome (Tabet et al., 2016). DGKε regulates seizure susceptibility and long-term potentiation (Rodriguez De Turco et al., 2001). DGKζ negatively regulates T-cell response (Zhong et al., 2003). In addition, DGKζ is involved in the maintenance of spine density (Kim et al., 2009) and reciprocally regulates p53 and nuclear factor-κB (Tanaka et al., 2013, 2016; Tsuchiya et al., 2015). DGKι inhibits Ras guanylnucleotide-releasing protein (GRP) 3-dependent-Rap1 signaling (Regier et al., 2005). DGKθ is suggested to be

Please cite this article in press as: Sakane, F., et al., Where do substrates of diacylglycerol kinases come from? Diacylglycerol kinases utilize diacylglycerol species supplied from phosphatidylinositol turnover-independent pathways, Advances in Biological Regulation (2017), http://dx.doi.org/10.1016/j.jbior.2017.09.003