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Review

Phospholipases C and sphingomyelinases: Lipids as substrates and modulators of enzyme activity

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ABSTRACT

This review article deals with phospholipases C (PLC), sphingomyelinases (SMases) and related lipases. Bacterial PC-preferring PLC and PI-specific PLC, bacterial SMases and PLC/SMases, eukaryotic SMases and ceramide phosphorylinositol hydrolases are discussed. The aim of the review is to offer a coherent description of lipid–protein interactions for the above enzymes, considering that (a) the enzyme activity is influenced by the physical properties of the substrate lipid, (b) the enzyme activity is modulated by non-substrate lipids, (c) enzyme end-products often change the physical properties of the lipid matrix, hence the enzyme activity. This approach allows a certain degree of understanding of phenomena such as: latency periods (lag times), enzyme interfacial activation, effects of intrinsic lipid curvature and of overall bilayer curvature on enzyme activity, and enzyme-promoted vesicle aggregation and fusion.

Contents

Abbreviations: aSMase, acid sphingomyelinase; Cer, ceramide; Chol, cholesterol; DAG, diacylglyceride; DPPC, dipalmitoyl phosphatidylcholine; FRET, fluorescence resonance energy transfer; GPI, glycosyl phosphatidylinositol; GUV, giant unilamellar vesicles; IP3, 1,4,5-inositol trisphosphate; LmPLC, *Listeria monocytogenes* phospholipase C/sphingomyelinase; LUV, large unilamellar vesicles; nSMase, neutral sphingomyelinase; PA, phosphatidic acid; PC, phosphatidylcholine; PC–PLC, phosphatidylcholine–preferring phospholipase C; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PH, plekstrin homology (domain); PI, phosphatidylinositol; PI–PLC, phosphatidylinositol–specific phospholipase C; PIP2, phosphatidylinositol-4,5-bisphosphate; PI-PLC, phospholipase C; PMe, phosphatidylmethanol; PS, phosphatidylserine; γSA, gamma-specific arrangement (in phospholipase Cγ); SM, sphingomyelin; SMase, sphingomyelinase; SUV, sonicated unilamellar vesicles.

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1. Introduction

Phospholipases C (PLCs) are phosphodiesterases that hydrolyse the phosphodiester bond between glycerol and phosphate in glycerophospholipids, yielding diacylglycerol (DAG) and a phosphoryl-base, e.g. phosphorylcholine, or phosphorylinositol. Sphingomyelinases (SMases) catalyse a similar reaction in sphingophospholipids, yielding ceramide (Cer) and a phosphoryl-base, phosphorylcholine when the original lipid is sphingomyelin (SM). Some authors refer to the latter enzymes as "sphingomyelinases C", keeping the name "sphingomyelinases D" for those enzymes that cleave the phosphodiester bond between phosphate and choline in SM. In this review we consider "sphingomyelinase" as synonym of "sphingomyelinase C".

Both PLCs and SMases have in common with the other lipases that, unlike most other enzymes, their substrate does not occur in solution, but rather in the aggregated state. This has a number of consequences that render the kinetics and regulation of these enzymes rather unique phenomena. Because of the extreme mesomorphism of the lipids (they can occur in several lamellar phases, plus the micellar, tubular or hexagonal, the various cubic phases, etc.) the substrate can exhibit very different physical properties, and the latter may in turn influence the enzyme activity. Moreover some of the reaction end-products of PLCs and SMases, respectively DAG and Cer, are also lipids but exhibit physical properties very different from those of the parent phospholipids, with the result that the enzyme activity is modifying the physical state of the substrate, which in turn will influence any further enzyme activity. Thus, lipase–substrate interactions are extremely intricate, and this review intends to clarify some of them.

The enzymes that are the object of this review were not long ago considered as mere instruments in lipid degradation. However, the discovery of the potent biological activities of DAG and Cer in the last decades has elicited a renewed interest in PLCs and SMases and their roles in cell signalling. For basic information on PLCs the reader is referred to the reviews by Titball [229] and Popoff and Bouvet [177], for bacterial PLCs, and by Suh et al. [221], Fukami et al. [65], and Bunney and Katan [17], for eukaryotic PLCs. Excellent reviews on sphingomyelinases have been published by Samet and Barenholz [197], Zeidan and Hannun [261], and Clarke et al. [33]. We also published a review on SMases [75], of which the present contribution is in part an update.

Because of the many biological effects of DAG and Cer, a large fraction of the literature on PLCs and SMases is devoted to their physiological or pathophysiological effects in the cell. This review does not cover those aspects, but is rather limited to the interplay between enzymes on one side, and lipids (substrates and end-products) on the other. An elementary knowledge of the phase behaviour of lipids, such as described in Feigenson [59], van Meer et al. [239], de Kruijff [45], is presupposed on the part of the reader. A summary of the physical properties of DAG and Cer, and of their influence on the bilayer properties is given below.

2. The properties of DAG and Cer in lipid bilayers

2.1. DAG

The structure and functional properties of DAG in membranes are described in detail in [72]. What follows is just a brief summary.

(a) 1,2-Diacyl-*sn*-glycerols (DAG) are minor components of cell membranes (about 1 mol% of the lipids) and yet they are potent regulators of both the physical properties of the lipid bilayer and the catalytic behaviour of several membrane-related enzymes.
(b) DAG mix with phospholipids in fluid bilayers when their melting temperature is below or close enough to the melting temperature of the bilayer system. When incorporated in phospholipid bilayers, the conformation of DAG is such that the glycerol backbone is nearly perpendicular to the bilayer, with the *sn*-1 chain extending from the glycerol C1 carbon into the hydrophobic matrix of the bilayer and the *sn*-2 chain first extending parallel to the bilayer surface, then making a 90° bend at the position of the *sn*-1 carbonyl to become parallel to the *sn*-1 chain. This conformation is similar to what is observed for most phospholipids. DAGs are located in phospholipid bilayers about two CH_2 units deeper than the adjacent phospholipids.
(c) DAGs mix nonideally with phospholipids, giving rise to in-plane separations of DAG-rich and -poor domains, even in the fluid state. DAG molecules also increase the separation between phospholipid headgroups, and decrease the hydration of the bilayer surface.
(d) Also, because the transversal section of the DAG headgroup is small when compared to that of the acyl chains, DAG favours the (negative) curvature of the lipid monolayers, and DAG-phospholipid mixtures tend to convert into inverted nonlamellar hexagonal or cubic phases.