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Review

Sphingomyelin metabolism at the plasma membrane: Implications for bioactive sphingolipids

Delphine Milhas, Christopher J. Clarke, Yusuf A. Hannun *

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, 173 Ashley Ave., Charleston, SC 29425, USA

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ABSTRACT

The plasma membrane (PM) is a major resource for production of bioactive lipids and contains a large proportion of the cellular sphingomyelin (SM) content. Consequently, the regulation of SM levels at the PM by enzymes such as sphingomyelinase (SMase) and SM synthase 2 (SMS2) can have profound effects – both on biophysical properties of the membrane, but also on cellular signaling. Over the past 20 years, there has been considerable research into the physiological and cellular functions associated with regulation of SM levels, notably with regards to the production of ceramide. In this review, we will summarize this research with particular focus on the SMases and SMS2. We will outline what biological functions are associated with SM metabolism/production at the PM, and discuss what we believe are major challenges that need to be addressed in future studies.

1. Introduction

The plasma membrane (PM) of the cell has the important function of separating the inner cellular environment from the extracellular space. However, it also has a crucial role in communication between cells and their environments, being involved in processes such as subcellular trafficking, signal transduction, and metabolite exchange. Like all membranes, the PM is comprised of a lipid bilayer with a hydrophobic core and hydrophilic surfaces – in this case, an extracellular surface and a cytosolic surface. Although conceived primarily (but not exclusively) as a single homogeneous domain in the classic fluid mosaic model [1], the PM is now thought to contain membrane microdomains termed 'lipid rafts'. Comprised of laterally segregated sphingomyelin (SM) and cholesterol tightly packed into liquid-ordered domains that exist in the surrounding 'liquid disordered' phase, it has been suggested that rafts have an important role in signal transduction and other cellular processes. This is discussed in more depth in the following reviews [2,3]. However, despite the abundance of literature investigating rafts, their existence and functional roles remain an issue of heated discussion.

As in most organelles, there is also an asymmetry within the PM lipid bilayer such that the compositions of the extracellular and cytosolic leaflets are significantly different. The outer leaflet is reported to be enriched in phosphatidylcholine (PC), SM and glycosphingolipids whereas the inner leaflet is abundant in phosphoinositides, phosphatidylserine (PS), phosphatidylethanolamine and phosphatidic acid [4,5]. Classically, the PM cholesterol is thought to be portioned relatively evenly between the two layers [5]; however, a recent study suggests the preponderance of cholesterol localizes to the inner leaflet [6]. Thus, with such distinct lipid environments, it is not difficult to imagine that metabolism of lipids within each leaflet may produce distinct physiological outcomes.

The PM contains a large portion of the cellular SM [7]. Not only is it a component of lipid rafts, but SM can be metabolized to ceramide, a bioactive lipid in its own right, but also a precursor molecule to other signaling lipids and a central hub of the sphingolipid network (reviewed in [8]). Conversely, the production of SM from ceramide and PC by the enzyme SM synthase can serve to regulate signal transduction through reducing ceramide levels, but also increasing levels of DAG and SM. Thus, both sphingomyelinase (SMases) and SM synthases can act as signaling switches at the PM. Moreover, as SM and ceramide have differing properties, the metabolism of SM to ceramide or production of SM from ceramide can consequently also have important biophysical effects on the membrane itself. Here, we will outline the current literature

Abbreviations: bSMase, bacterial sphingomyelinase; LDL, low density lipoprotein; L-SMase, lysosomal acid sphingomyelinase; N-SMase, neutral sphingomyelinase; PC, phosphatidylcholine; PLC, phospholipase C; PM, plasma membrane; SK, sphingosine kinase; SM, sphingomyelin; SMase, sphingomyelinase; SMS1, sphingomyelinase synthase 1; SMS2, sphingomyelin synthase 2; S-SMase, secretory sphingomyelinase

* Corresponding author. Fax: +1 843 792 4322.
E-mail address: hannun@musc.edu (Y.A. Hannun).

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related to SM metabolism at the PM with particular focus on the SMases and sphingomyelin synthase 2 (SMS2). In addition, we will review the biological functions associated with SM metabolism/production at the PM, and will briefly discuss the major challenges to be addressed in the future.

2. Bacterial sphingomyelinase

Many pathogenic bacteria produce proteins with neutral SMase activity despite the fact that they are unable to synthesize sphingolipids themselves. The bacterial sphingomyelinases (bSMases) are secretory proteins and are part of a larger family of phospholipases released into the environment where they are able to utilize host cell lipids to exert cytotoxic effects [9]. Importantly, the identification and purification of bSMases provided the sphingolipid field with useful tools with which to study the effects of endogenous ceramide generation through exogenous addition of bSMases to cell culture, a practice still in use today. However, it should be cautioned that the effects of exogenous bSMases on PM lipids can extend beyond SM, causing hydrolysis of other membrane lipids such as PC. Thus, these effects should be taken into account when interpreting results.

2.1. Properties

Currently, bSMase genes have been identified from a number of bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Listeria ivanovii*, *Leptospira interrogans* and the *Pseudomonas* sp. Strain TK4. In addition, bSMase activities have been characterized from *Helicobacter pylori* and *Mycobacterium tuberculosis* (see [10] and references therein). It should also be noted that a number of other members of the bacterial phospholipase family often possess both phospholipase C (PLC) and SMase activities such as PlcHR from *P. aerugonosa* [11], α-toxin from *Clostridium perfringens* [12], and PlcB from *Listeria monocytogenes* [13]. Many of the identified bacterial SMases share common properties with molecular masses of between 33 and 38 kDa although there are exceptions such as the bSMases from TK4 (58 kDa) and *Le. interrogans* (63 kDa) (see [10]). The SMase activity of all these proteins has neutral pH optima and is dependent on divalent cations such as Mg^{2+} and Mn^{2+}, whereas Ca^{2+} and Sr^{2+} are both ineffective for hydrolysis [14]. Based on the homology of the active site residues, the bSMases were classified as members of the DNAse 1 superfamily and this was subsequently confirmed with the recent solving of the crystal structures of bSMases from *B. cereus*, *L. ivanovii* and β-toxin from *S. aureus* [14–16]. These structures also shed light on the catalytic mechanisms, and it was proposed that SM hydrolysis proceeds by acid–base catalysis through a pentavalent phosphorus transition state. For a more detailed description of the proposed mechanism, the reader is referred to [14]. Notably, the conserved catalytic site residues throughout many of the N-SMases in higher organisms suggest a common catalytic mechanism. Finally, bSMases from *B. cereus*, *S. aureus* and *L. ivanovii* all contain a hydrophobic β hairpin structure that is necessary for interaction of the bSMases with cell membranes and liposomes and, presumably, functions to bring the SMase into close proximity to its substrate on the outer membrane [14]. However, it should also be noted that this region is not wholly conserved in the bSMases from *Le. interrogans*, the *Pseudomonas* sp. strain TK4 or *S. epidermis* which suggests that there are additional mechanisms for bSMase interaction with the membrane [15,16].

2.2. Bacterial SMase and hemolysis

The best characterized action of bSMases is their ability to induce hemolysis – the breaking open of red blood cells with subsequent release of hemoglobin into the surrounding fluid. Thus far, hemolytic activity has been reported for all known bSMases [9,10] and many studies have observed that the susceptibility of erythrocytes to undergo hemolysis correlates with their cellular SM content i.e. sheep erythrocytes (~50% SM) readily undergo bSMase-induced hemolysis whereas, for example, horse erythrocytes (~10% SM) are much more resistant [9]. In addition, kinetic analysis of the bSMase from *Staphylococcus schleiferi* found that the SMase activities and hemolytic activities were closely related and showed identical Michaelis–Mentin kinetics [17]. Thus, it was inferred that SMase activity plays a crucial role in hemolysis. Subsequent confirmation of this came from genetic studies of the β-toxin from *S. aureus* where it was found that mutation of key catalytic residues abolished SMase activity, and prevented all hemolytic activity [16]. Moreover, similar studies in α-toxin from *C. perfringens* and $PlcHR_2$ from *Pseudomonas aeruginosa*, proteins that possesses both PLC and SMase activities, have found that the SMase activities of these enzymes are more crucial for hemolysis than the PLC activities [12,18]. Taken together, these results suggest that bSMase-mediated hydrolysis of SM at the PM is crucial for their hemolytic activity.

It is well established that the sensitivity of erythrocytes to hemolysis to certain agents increases when cells are cooled to 4 °C following incubation with the hemolytic agents at 37 °C [18] although the mechanism underlying this phenomenon termed 'hot–cold hemolysis' has remained unclear. Given the crucial role of SMase activity in hemolysis as described above, it was suggested that SM metabolism at the membrane is important for hot–cold hemolysis. Indeed, an early study postulated that such SM hydrolysis generated 'fragile' erythrocytes so that, on cooling, the altered membrane properties caused sufficient stress to result in the lysis [19]. More recent studies have begun to probe this question further. A recent study that analyzed erythrocyte ghost membranes following hemolysis induced by $PlcHR_2$ from *P. aeruginosa* and found the presence of large ceramide-rich domains; importantly, these domains were fluid at 37 °C but rigid at 4 °C [18]. Moreover, ceramidase treatment reduced the presence of these domains and, consequently, reduced hot–cold hemolysis induced by $PlcHR_2$ [18]. Taken together, these results indicate that the formation of ceramide-rich domains by a bacterial SMase activity plays an important role in the phenomenon of hot–cold hemolysis.

2.3. Bacterial SMase and other mammalian cell types

In addition to their deleterious effects on erythrocytes, studies have reported that bSMases are also cytotoxic to other mammalian cell types albeit to varying degrees. An early study found that bSMases from *S. aureus*, *B. cereus*, and *Streptomyces* sp. were selectively cytotoxic to human monocytes yet had little effects on viability of human granulocytes, fibroblasts, or lymphocytes despite being able to degrade SM efficiently in these cells [20]. In contrast, studies with Sph2 from *Le. interrogans* reported cytotoxic effects on mouse lymphocytes, mouse macrophages, and human liver cells in addition to its hemolytic properties [21] while a more recent study with SMase (β-toxin) from *S. aureus* reported cytoxicity against proliferating T-cells; importantly, both hemolysis and leukotoxicity were dependent on enzymatic activity [16]. Moreover, $PlcHr_2$ from *P. aeruginosa*, which is both a SMase and PC-PLC, was reported to be selectively cytotoxic to endothelial cells yet was weakly cytotoxic to HeLa and A549 cells [22]. Notably, the cytotoxic effect of $PlcHr_2$ on endothelial cells resulted in suppression of angiogenesis; however, it was not clear which enzymatic activities were important for this effect [11]. In contrast to this, beta-hemoylsin from *S. aureus* was found to suppress production of interleukin-8 from endothelial cells, but did not have effects on cell viability; with regards to the former effect, this was postulated to be a result of SM