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Sphingomyelin: What is it good for?

Félix M. Goñi

Instituto Biofisika (CSIC, UPV/EHU) and Department of Biochemistry, University of the Basque Country, 48940, Leioa, Spain

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ABSTRACT

Sphingomyelin has been considered as a merely structural lipid for many years. However, this organelle-specific lipid has many other roles, including increasing membrane molecular order, acting as a source of ceramide in cell signaling and apoptosis, and forming clusters/nanodomains with cholesterol and ceramide. This contribution is dedicated to Professor E. Carafoli, on occasion of his 90th anniversary.

1. An enigmatic lipid

For many decades, lipids have occupied an obscure corner in the gallery of biomolecules. Their structure was perceived as a monotonous series of methylene (−CH_2−) groups, their role limited to "depot fat", or at most to components of cell membranes, the latter understood as biological brick layers. Even for biochemists and molecular biologists the word "lipid" has the negative connotation of "fats", something to be avoided at all cost in contemporary society. Few of our colleagues in the medico-biological community appear to realize the purely lipidic nature of delicate yet highly active structures such as the steroid hormones, several vitamins, or the prostanoids.

Focusing on the membrane lipids, the vast majority (glycerolipids) consists of derivatives of the polyalcohol glycerol. Only a fraction that rarely exceeds 5% of the total membrane lipid, the sphingolipids, is structurally based on sphingosine, a molecule bearing both alcohol and amino groups. The name sphingolipids was selected by their discoverer J.L.W. Tudichum, in 1884. Because of their enigmatic nature, he named them after the Sphinx, a Greek mythological creature famous for posing difficult questions, or enigmas. Their main representative, sphingomyelin (SM), was seen as a merely structural component in the myelin sheaths.

Sphingomyelin (SM) is one of the main phospholipids that make up the hydrophobic matrix of mammalian membranes. For decades it was considered as a merely "structural" lipid. Its abundance in the relatively inactive myelin sheath membranes supported this view. However, the discovery in the 1980s of the sphingolipid signaling pathway, in which SM is cleaved by a sphingomyelinase in response to a stress condition, brought a new light on this phospholipid [1]. The present contribution discusses the multiple role of SM, as a stable membrane building block, and as generator and regulator of powerful metabolic signals.

2. A lipid that brings stability to membranes

Phospholipids tend to self-organize spontaneously in lamellae formed by a double layer (bilayers) that in turn contribute the basic structure of biological membranes [2]. Not all membrane phospholipids have the same propensity to form lamellar structures. Depending on the approximate geometric shape of their molecules, the cylindrical ones (e.g.phosphatidyl choline) readily give rise to flat bilayers. Other lipids however exhibit a conical geometry (e.g. phosphatidyl ethanolamine) and they cannot generate spontaneously a bilayer. In fact these conical lipids are responsible for a certain degree of instability in the cell membranes, which make use of this property to respond to a variety of stimuli. SM belongs to the "cylindrical" lipids that contribute to the geometrical stability of the cell membranes.

SM is also a particularly stable phospholipid from the biochemical point of view. The usual phospholipids (phosphatidyl choline, phosphatidyl ethanolamine) are glycerolipids, i.e. they are structurally arranged as derivatives of glycerol. Glycerophospholipids are susceptible to attack by several kinds of phospholipases (A_2, C, D). SM however does not contain glycerol. Its structural basis is a long-chain alcohol, sphingosine, which occurs with minor variations in all sphingolipids. SM cannot be hydrolysed but by a single specific phospholipase, called sphingomyelinase. Sphingomyelinases are far less abundant than phospholipases in mammals, thus SM is biochemically more stable than most phospholipids.

Abbreviations: SM, sphingomyelin; Cer, ceramide.

E-mail address: felix.goni@ehu.eus.

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3. A lipid of order

The degree of *molecular order* in the membrane lipid hydrocarbon chains, also related to the concept of membrane fluidity, is an important parameter that can be quantified with various spectroscopic techniques. Membrane order influences greatly the activity of membrane-bound enzymes and many other cell physiology processes. SMs increase membrane order because their melting (gel-to-fluid) temperature is usually above 37 °C (depending on its N-acyl chain), thus their spontaneous tendency at the physiological temperature is to stay in the rigid, gel phase, tending to order the hydrocarbon chains.

In addition to being by itself a lipid promoting molecular order in membranes, SM associates with cholesterol, the chief inducer of molecular order, because of its flat, rigid structure. The main driving force for the close SM-cholesterol interaction seems to be a hydrogen bond generated by the amide group of the SM molecule and the 3-hydroxyl group of cholesterol. The SM-cholesterol mixture, in aqueous media, gives rise to a particular form of lamellae, said to be in the *liquid-ordered phase* [3], as opposed to the usual liquid-disordered, or fluid phase. (Certain saturated glycerophospholipids can also give rise to a liquid-ordered phase with cholesterol, although the stabilizing intermolecular interactions are weaker in the latter case). Under certain conditions, *domains* (i.e. segregated regions of lipids) in the liquid-ordered phase tend to separate from the liquid-disordered ones. The existence of liquid-ordered nanodomains has been associated to the much-debated existence of membrane "rafts". Experimental evidence confirms the preference of a variety of membrane proteins for liquid-ordered micron-sized domains, at least in model systems.

4. A lipid that can release a hidden beast

Even if SM is a particularly stable lipid from the biochemical point of view, and one that provides physical stability to cell membranes, it is also a major source of one of the most potent bioactive lipids, ceramide (Fig. 1). There are numerous examples of cells that, under stress conditions, secrete an acid sphingomyelinase that in turns hydrolyses SM in the plasma membrane outer leaflet, yielding ceramide (Cer) [4]. The latter is considered to be an inductor of apoptosis. In addition, it has been shown to break down the membrane permeability barrier and to destroy the bilayer asymmetry, among other properties. Also ceramide is a precursor of sphingosine, another highly bioactive lipid. Thus Cer is a metabolic

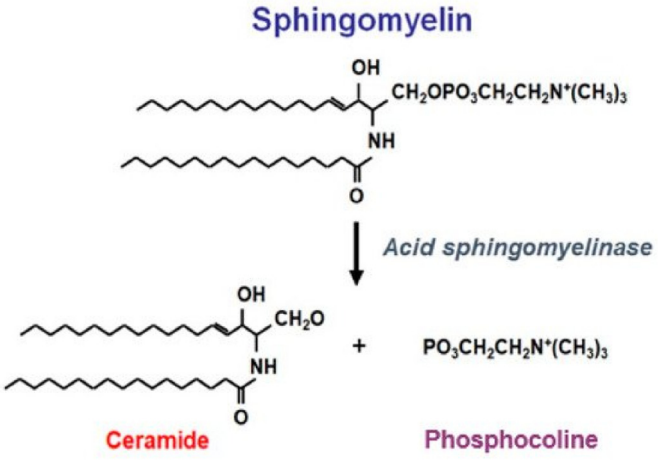

Fig. 1. SM hydrolysis to yield P-choline and ceramide.

wild beast in contrast with its largely inert precursor SM.

5. A lipid that tames the beast

Not only is SM a precursor of Cer, it also binds Cer with great affinity. Cer has a great affinity for SM even in the presence of other lipids, and tends to form domains enriched in SM-Cer [5]. The presence in the SM and Cer polar headgroups of H-bond donors and acceptors is the probable explanation for the formation of SM-Cer domains, stabilized by a network of H-bonds between the polar headgroups. Thus when as a result of a stress signal part of the SM in a membrane is degraded to Cer, only a small proportion of the latter remains free and available for its physical and biochemical activities, most of it remains SM-bound, and probably little active. Among other unique properties, Cer display very high gel-fluid transition temperatures, often above 80 °C. Domains enriched in SM-Cer are so much more rigid than the surrounding bilayer that they exist in the gel (solid-ordered) phase at 37 °C. An interesting feature of the SM-Cer gel phase in binary systems (without cholesterol) is that it seems to be stoichiometrically constant. Thus SM acts both as a precursor and a *buffer* of Cer activity.

The situation is made more complex, i.e. interesting, in the presence of the ternary system SM, Cer and cholesterol (Fig. 2). In those bilayers the existence of Cer-enriched domains is strongly dependent on cholesterol content and this would support the idea of a critical balance between Cer and cholesterol levels in membranes as powerful modulators of the activation of signaling processes such as apoptosis. The coexistence of two distinct phases (respectively SM-cholesterol-enriched and SM-Cer-enriched) has been confirmed by various techniques. When Cer displaces cholesterol, the gel phase formed by Cer and SM has a higher packing than the original liquid-ordered phase, thus its nanomechanical properties and thickness are strongly affected. In turn, when the lipid system is highly saturated in cholesterol, Cer is not able to displace the former. Moreover, a high concentration of cholesterol prevents Cer-enriched domain segregation in fluid and in non-fluid membranes, i.e. SM systems highly saturated in both cholesterol and Cer. This demonstrates that Cer-cholesterol displacement is not a matter of a special preference or interaction of any of them towards SM (or any other phospholipid), but rather a matter of the relative ratio of both molecules, as both have a tendency (due to their hydrophobicity) to occupy the spaces between the lipid tails of phospholipids. It has been shown that SM, Cer and cholesterol are actually able to coexist in a single ternary gel phase (54:23:23 mol ratio) with intermediate properties between SM-Cer-enriched domains and cholesterol-driven liquid-ordered phases, and that the phase would be stabilized by direct Cer-cholesterol interactions [6]. The fact that Cer-cholesterol ratios govern the properties of this SM-rich ternary phase suggests that SM would govern a dynamic mechanism through which cells could modulate the biophysical properties of their membranes according to their needs, including assembly and disassembly of liquid-ordered phases, molecular packing, membrane order or thickness.

6. An organelle-specific lipid

Unlike most phospholipids, including the structurally-related phosphatidyl choline, SM is not distributed more or less equally among the organelle membranes, rather it is found mainly in the plasma membrane, the *trans*-Golgi network, and the lysosome [4]. In the plasma membrane SM is located almost exclusively in the outer monolayer, evoking its role as an inert, structural lipid. But it acts also there as the substrate for acid sphingomyelinase, secreted from the cytosol to degrade SM and liberate Cer in response to stress. In the Golgi membranes, liquid-ordered SM-rich domains

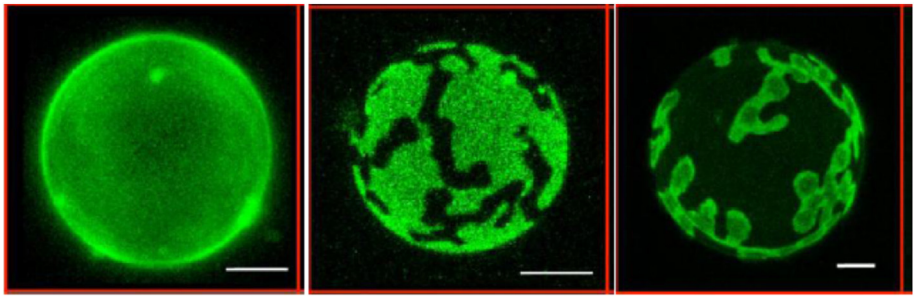

Fig. 2. Confocal microscopy of DiIC18-stained SM/cholesterol/ceramide (54:23:23 mol ratio) (left), SM/cholestenone/ceramide (middle), SM/cholesterol/dipalmitoyl glycerol (right). Only in the presence of SM, cholesterol and ceramide is a single phase observed. Temperature: 22 °C. Bars: 5 μm [6].

are essential in the formation of functional enzyme domains, but not for the fusion of incoming transport carriers. The presence of SM would lead to the separation of domains of different thickness, commensurate with the length of the protein transmembrane domains. Such domains would help to increase the local concentration of enzyme-substrate couples. In the lysosome in turn, the presence of SM mainly originates from degraded Golgi fragments, but the phospholipid becomes important in the pathogenesis of diseases (Niemann-Pick and related) due to defective lipid degradation.

7. Concluding remarks

SM, traditionally perceived as a simply structural lipid in low-activity membranes, has become a central molecule in multiple cell signaling events. SM thus joins an increasing number of biomolecules, from non-coding DNA to 'depot fat', long considered as structural, for which important other functions are being discovered. Perhaps it is a general trend of evolution to avoid purely static structures when they can be given an additional use.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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