Biochimica et Biophysica Acta 1858 (2016) 304–310

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

The importance of hydrogen bonding in sphingomyelin's membrane interactions with co-lipids☆

J. Peter Slotte

Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 16 November 2015
Accepted 3 December 2015
Available online 4 December 2015

Keywords:
Sphingomyelin
Cholesterol
Ceramide
Hydrogen bond
Bilayer
Computational modeling

ABSTRACT

Sphingomyelin is an important constituent of mammalian cell membranes. Its molecular structure is *N*-acyl-D-*erythro*-sphingosylphosphorylcholine. The *N*-acyls in sphingomyelin often contain 16–24 carbons that are mostly saturated chains; however, the monounsaturated 24:1$^{\Delta 15c}$ acyl chain is also common. In addition to the more saturated nature of sphingomyelins, compared to physiologically relevant glycerophospholipids, also their hydrogen bonding properties are very different from the glycerophospholipids. Sphingomyelins form extensive intramolecular hydrogen bonds (from the 3OH of the long-chain base to phosphate oxygens of the head group), but also intermolecular hydrogen bonding involving the NH of the long-chain base are important for sphingomyelin (and sphingolipid) properties in membrane environments. Hydrogen bonding involving sphingomyelin has been shown to markedly stabilize interactions with both cholesterol and ceramide in fully hydrated bilayers. Such interactions contribute to the propensity of saturated sphingomyelin to form a liquid-ordered phase together with cholesterol, or a gel phase with saturated ceramides. The purpose of this review is to present recent experimental and computational evidence in support of the importance of hydrogen bonding for the interaction of sphingomyelin with other membrane lipids.

Contents

1. Introduction . . . 304
2. Intramolecular hydrogen bonding in SM . . . 305
3. Intermolecular hydrogen bonding in SM . . . 306
4. Role of hydrogen bonding in SM's association with ceramide . . . 306
5. Role of hydrogen bonding in cholesterol's association with SM . . . 307
Conflict of interest . . . 309
Acknowledgments . . . 309
References . . . 309

1. Introduction

As a broad definition, "a hydrogen bond is said to exist when (*i*) there is evidence of a bond, and (*ii*) there is evidence that this bond sterically involves a hydrogen atom already bonded to another atom" [1]. Some hydrogen bonding is evidenced by hard facts (such as hydrogen bonding between water molecules in liquid and ice [2]), but for biological membranes, evidence of hydrogen bonding between lipids is often determined indirectly and by inference. However, in general, scientists agree that hydrogen bonding must be important for stabilizing molecular interactions in biological membranes.

The main functional groups that are present in biological macromolecules and lipids and that can be involved in hydrogen bonding are shown in Fig. 1 [3]. Hydrogen bonds in biological systems are almost always weak, with bond energies below 20 kJ/mol. They are weakly directional (donor-acceptor direction within 160 ± 20°), and are often multicentered. The strength of the hydrogen bond falls off with r^{-1}, which means it may stabilize interactions between atoms at fairly long distances

Abbreviations: DOPC, 1-oleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DPH-SM, *N*-propionyl[diphenylhexatriene]-D-*erythro*-sphingosylphosphorylcholine; DPPC, 1-palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; FTIR, Fourier transform infrared spectroscopy; GUV, giant unilamellar vesicle; L_o, liquid-ordered phase; NMR, nuclear magnetic resonance spectroscopy; PCer, *N*-palmitoyl-D-*erythro*-sphingosine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PSM, *N*-palmitoyl-D-*erythro*-sphingosylphosphorylcholine; PSPC, 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; SM, sphingomyelin; SSM, *N*-stearoyl-D-*erythro*-sphingosylphosphorylcholine.

☆ In memory of professor Bob Bittman.

E-mail address: jpslotte@abo.fi.

http://dx.doi.org/10.1016/j.bbamem.2015.12.008
0005-2736/

(e.g., 3.5 Å for H···O). In this review, "hydrogen bond" is used as a general term, implying attractive interactions mainly involving the 2NH and 3OH functional groups of sphingomyelin (SM). In many cases, especially for interlipid hydrogen bonding, the interacting functional groups in adjacent molecules are not always known, at least based on experimental evidence. Sometimes, hydrogen bonding may imply intermediate water bridges. For comprehensive reviews on intermolecular hydrogen bonding among lipids in bilayer membranes, please see Boggs [4,5].

SM is a common phospholipid in the membranes of mammalian eukaryotic cells. It was first isolated and characterized by Thudicum [6], who showed that its degradation products included fatty acid, phosphoric acid, choline, and a second base with an unknown structure. The structure of the unknown base (now known as sphingosine) was determined by Carter and coworkers in 1947 [7]. Rouser and colleagues determined that the phosphocholine head group of SM was linked to the hydroxyl on C1 of sphingosine [8]. The final proof for the chiral conformation of 2NH and 3OH in natural SM was determined by Shapiro and Fowlers in 1962 [9] (the structure of *N*-palmitoyl-SM [PSM] is shown in Fig. 2). The long-chain base of SM is often (2S,3R)-2-aminooctadec-4-ene-1,3-diol (sphingosine), but in many SM species it may vary in length and may be additionally hydroxylated (4-OH in phyto-SM), methyl-branched, or fully saturated (in dihydro-SM). The *N*-linked acyl chain is often fully saturated (C16:0, C18:0, and C24:0 are common acyl chains), but it can also be monounsaturated (typically C24:1Δ^{15c}). In specialized cells, much longer polyunsaturated acyl chains *N*-linked to the long-chain base of SM may also exist [10]. Comprehensive reviews of SM's chemistry, biophysics, and biology are available [11–14].

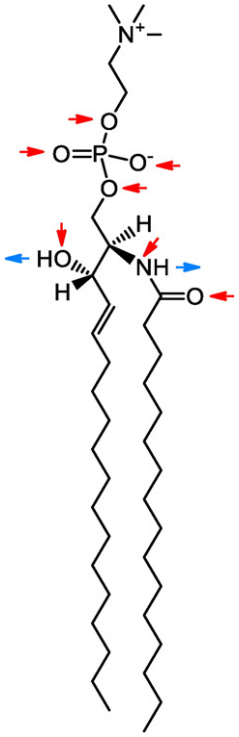

Fig. 2. Molecular structure of *N*-palmitoyl-D-*erythro*-sphingosylphosphorylcholine. Hydrogen-bond-donating groups are marked with blue arrows, and hydrogen-bond-accepting groups are indicated with red arrows.

2. Intramolecular hydrogen bonding in SM

In what was probably the first systematic structural study on the difference between SM and phosphatidylcholine (PC) bilayers, Schmidt, Barenholz, and Thompson showed, using ^{1}H and ^{31}P nuclear magnetic resonance (NMR), that the temperature variation of proton line widths and the spin–lattice relaxation times differed between the SM and PC bilayers [15]. Because both SM and PC have the phosphocholine head group, the measured difference was interpreted to be due to intramolecular hydrogen bonding, which is more extensive in SM bilayers than in PC bilayers. It had previously been suggested that SM probably hydrogen-bonds differently than PC because of structural differences [16]. Examining the head-group conformations of SM using high-resolution NMR, Bruzik suggested that a hydrogen bond between the 3OH of the long-chain base and phosphate oxygen could explain the stable conformer found for D-*erythro*-SM, which was not seen in the L-*threo* isomer [17]. Almost simultaneously, using Fourier transform infrared spectroscopy (FTIR) to compare the stretching frequencies originating from the phosphate group, Villalain and coworkers concluded that the differences seen in SM and PC bilayers suggested the presence of intra- and intermolecular hydrogen bonding in the SM bilayers [18]. They further suggested

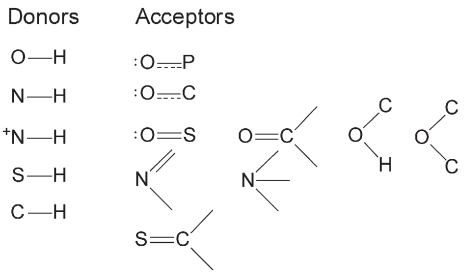

Fig. 1. Typical functional groups of biological molecules involved in hydrogen bonding. Adapted from Jeffrey and Saenger [3].

that the 3OH in the long-chain base of SM was the most likely a hydrogen-bonding donor linking the phosphate to the interfacial region. However, in another FTIR and Raman spectroscopy study [19], it was suggested that the 3OH formed a strong intermolecular hydrogen bond, probably to carbonyl oxygens in adjacent molecules. This study also suggested that the 2NH group was the donor in forming intermolecular hydrogen bonds. Further ^{1}H and ^{31}P NMR studies by the same group provided data supporting the existence of intramolecular hydrogen bonding between the 3OH and phosphate oxygen [20]. This hydrogen bond was present both in monomeric SM and in aggregated forms of SM. Interestingly, the hydrogen bond formed from the 3OH was interpreted to be stronger in SM than in dihydro-SM (which lacks the C4-5 *trans* double bond of the long-chain base), suggesting that this unsaturation somehow affected the hydrogen-donating propensity of the 3OH [20]. A ^{31}P NMR study comparing the head-group motion in SM with PC bilayers suggested that the SM head group experienced both intra- and interlipid hydrogen bonding, which was not seen with head-group motion in the PC bilayers [21]. This was true both in the disordered (L_α) and the ordered ($L_{\beta'}$) phase [21]. Further indirect proof for a role of hydrogen bonding from 3OH was seen with a 3-methoxy analog of SM, the gel-phase packing of which was significantly destabilized compared to a chain-matched non-methylated SM [22].

Many of the early atomistic simulation studies regarding SM bilayers suggested that intramolecular hydrogen bonding from 3OH and 2NH to phosphate oxygens (Fig. 3) was very likely, and frequently occurring in hydrated bilayers [23–30]. It was also found that the *trans* configuration of the sphingoid base double bond allowed for tighter packing and more extensive hydrogen bonding, compared to a *cis* analog [30]. Later simulation studies with improved force fields suggested that intramolecular hydrogen bonds form in 99% of cases between 3OH and the phosphate oxygens [31]. The rate of hydrogen-bond formation is lower for interlipid hydrogen bonding involving the 2NH [31].