Bioorganic & Medicinal Chemistry 23 (2015) 2989–2994

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Novel Raman-tagged sphingomyelin that closely mimics original raft-forming behavior

Jin Cui [a,b,c], Shigeru Matsuoka [a,b,c], Masanao Kinoshita [a,b,c,†], Nobuaki Matsumori [a,†], Fuminori Sato [b,c], Michio Murata [a,b,c,*], Jun Ando [d,e,f,g], Hiroyuki Yamakoshi [d,e], Kosuke Dodo [d,e,f], Mikiko Sodeoka [d,e,f]

[a] Department of Chemistry, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan
[b] JST, ERATO, Lipid Active Structure Project, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan
[c] Project Research Centre for Fundamental Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan
[d] JST, ERATO, Sodeoka Live Cell Chemistry Project, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[e] Synthetic Organic Chemistry Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[f] AMED-CREST, AMED 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[g] Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 1 April 2015
Revised 3 May 2015
Accepted 5 May 2015
Available online 12 May 2015

Keywords:
Sphingomyelin
Lipid rafts
Raman imaging
Solid-state ^{2}H NMR
Phase distribution

ABSTRACT

Three Raman probes of sphingomyelin (SM) were synthesized and evaluated for their applicability to imaging experiments. One probe containing a hydroxymethyl-1,3-butadiyne moiety in the polar head group showed strong scattering. The solid-state ^{2}H NMR spectra of this probe in oriented bilayer membrane revealed excellent compatibility with natural SM in phase behavior since the probe undergoes phase separation to form raft-like liquid ordered (L_o) domains in the raft-mimicking mixed bilayers.

1. Introduction

Lipid rafts show a similar character to the lipid ordered (L_o) phase in artificial membranes, which are often represented by microdomains enriched with sphingolipids (SM) and cholesterol (chol). The physicochemical properties of the specific lipids are believed to be important for fulfilling the raft functions such as cellular signal transduction.[1] To gain better insight into the role of lipid rafts in these processes, a direct optical visualization of such domains is essential. However, the task in raft imaging has not been truly achieved due to the lack of a suitable raft-specific probe. Fluorescent microscopy, which is commonly used for observing molecular localization, requires doping of the membranes with fluorescently labeled lipids. However, the probes attached with a relatively large fluorophore possess physical properties that are somewhat different from those of small lipid molecules, and often alter the membrane properties.[2] In model membranes, the chemical modification of a lipid molecule with a fluorescent moiety usually results in an inconsistent, or even completely opposite, distribution tendency in the membrane phases.[3]

In contrast, Raman-active moieties are considered to have less influence on the membrane properties due to their small size. The Raman image can be generated by measuring a specific molecular vibration to map the Raman-tagged molecules.[4] Functional groups such as diyne, azide, deuterium, and nitrile groups give rise to Raman scattering bands in the cellular silent region (1800–2800 cm^{-1}), where most endogenous biomolecules do not exhibit a signal.[5] Alkyne-tagged coenzyme Q (AltQ) and 5-ethynyl-2′-deoxyuridine (EdU) analogs have been recently used for imaging their distribution in living HeLa cells.[5] The most significant features of this tag are its suitable wavenumber of the Raman band and strong scattering intensity, which allow high-contrast imaging. Deuterium is expected to be another promising candidate due to its small size and good physical compatibility. Although the Raman intensity derived from the C–D bond is relatively weak, multiple-deuterated methylene or methyl groups may generate relatively strong Raman signals. Thus, we deduced that these tags could be further applied in raft-specific lipids to mark raft-like domains.

* Corresponding author. Tel./fax: +81 66850 5774.
E-mail address: murata@chem.sci.osaka-u.ac.jp (M. Murata).
† Present address: Department of Chemistry, Graduate School of Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8581, Japan.

http://dx.doi.org/10.1016/j.bmc.2015.05.014
0968-0896/

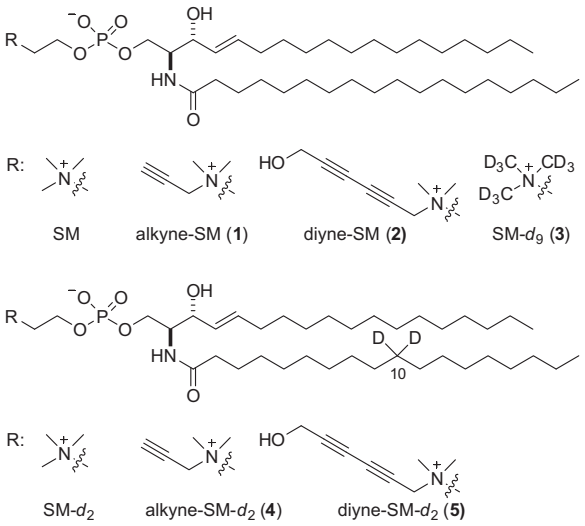


Figure 1. Chemical structures of natural SM, three Raman-tagged SMs and deuterated SMs discussed herein.

In this study, we designed and synthesized Raman-tagged SMs at the polar head, and measured their Raman spectra in a monolayer form. For promising candidates, we further evaluated their membrane behavior using ^{2}H solid state NMR with oriented bilayer membranes. Probes **1** and **2** possess terminal alkyne and hydroxymethyl-1,3-butadiyne groups, respectively; Probe **3** is labeled with nine deuterium atoms at the trimethyl ammonium moiety (Fig. 1), which encompasses the largest number of chemically equivalent C–D bonds in the polar head of SM. All of the probes possess a stearoyl (C18:0) moiety since stearic acid is one of the common acyl groups in SMs, particularly in those of bovine brain.[6]

In order to observe domain formation by Raman microscopy, previous studies have adopted acyl-labeled Raman probes such as DPPC-d_{62} or DSPC-d_{70}, in which the glycerophospholipid was substituted with perdeuterated fatty acids.[7] Although the acyl-deuterated probes are believed to have excellent compatibility with the natural lipids, the hydrocarbon packing in membrane are slightly different between highly deuterated and non-deuterated acyl chains.[7a,8] The lipid rafts are best characterized by the highly packed acyl chains, as is the case with the L_o phase in artificial membranes. A small but significant perturbation in the acyl chain behavior caused by deuteration may hamper us from elucidating the precise phase properties of the original lipid. In contrast, since the polar head of phospholipids is relatively loosely packed even in the L_o-phase, this portion is occasionally more suitable for chemical modifications. Thus, for investigating the atomistic mechanism underlying formation of the raft-mimicking L_o phase, besides acyl chain-tagged Raman probes, those with a small tag on the head group can be complimentarily used in the imaging of membrane domains.

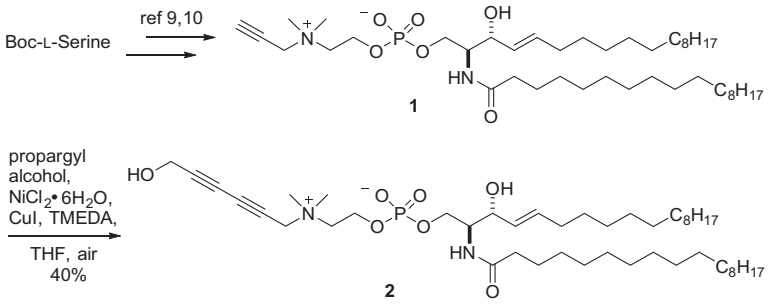
2. Results and discussion

Alkyne-SM **1** was first synthesized from Boc-L-serine through a protected sphingosine using a partially modified procedure from those in previous reports (Scheme 1).[9,10] Initially, we attempted to introduce the conjugated diyne moiety using 3-bromoprop-2-yn-1-ol as the coupling partner under the classical Cadiot–Chodkiewicz coupling conditions (copper(I) chloride, 5 mol %; hydroxylamine, 30 mol %; *i*-PrNH$_2$ in aqueous methanol),[11] as reported separately.[12] Unfortunately, it was difficult to drive the coupling to completion and only a modest conversion was achieved, as judged by ^{1}H NMR. Furthermore, the remaining alkyne **1** was hardly separable from compound **2** due to their similar polarities. Lei's group reported a mild Ni/Cu-cocatalyzed oxidative coupling reaction, by which the C_{sp}–C_{sp} bond formation was performed in favor of the heterocoupled product over the homocoupled products by increasing the molar ratio of the two terminal alkynes.[13] Based on their conditions, the coupling of **1** with a large excess of propargyl alcohol enabled selective conversion to **2**, which was easily separated by flash chromatography, although the isolated yield was modest (40%).

The synthetic route for SM-d_9 **3** is shown in Scheme 2. Substrate **6**, which was synthesized from Boc-L-serine,[9] was treated with N(CD$_3$)$_3$ in MeOH furnished **7** in a modest yield. Boc deprotection of **7** by TFA, followed by acylation with *p*-nitrophenyl stearate, produced the desired compound **3**.

The deuterated side chains of **4** and **5** were synthesized by adopting a more efficient route including a copper-catalyzed Kumada–Corriu coupling[14] as the key step compared to previously reported method,[15] (Scheme 3). Protection of the primary alcohol group in **8** afforded compound **9**. Reduction of the ester with LiAlD$_4$, followed by tosylation, provided deuterated intermediate **11**. The tosylated precursor has been considered as an efficient coupling partner for the C_{sp3}–C_{sp3} coupling.[16] Compound **11** was subsequently coupled with a Grignard reagent to afford **12** in excellent yield (91%). Removal of TBDPS, followed by Jones' oxidation, led to deuterated **14**, which was then converted into the corresponding active ester **15** by a condensation reaction with *p*-nitrophenol. Using the same synthetic method as for compound **1** and **2**, the deuterated samples **4** and 5 were synthesized.[17]

With these Raman-tagged SMs in hand, we first compared the relative Raman shift and intensity using their monolayer systems prepared by the Langmuir–Blodgett (LB) technique. As shown in Figure 2, the signals from Raman tags, such as diynes, alkynes, and C–D bonds, are observed in the cellular Raman-silent region, where no Raman band occurs from non-labeled SM, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) or chol; these three lipids are the constituents of raft-model membranes. The triple bond in



Scheme 1. Synthesis of alkyne-SM **1** and diyne-SM **2**.