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Synthetic lipids and their role in defining macromolecular assemblies

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ABSTRACT

Lipids have a variety of physiological roles, ranging from structural and biophysical contributions to membrane functions to signaling contributions in normal and abnormal physiology. This review highlights some of the contributions made by Robert Bittman to our understanding of lipid assemblies through the production of synthetic lipid analogs in the sterol, sphingolipid, and glycolipid classes. His contributions have included the development of a fluorescent cholesterol analog that shows strong functional analogies to cholesterol that has allowed live imaging of cholesterol distribution in living systems, to stereospecific synthetic approaches to both sphingolipid and glycolipid analogs crucial in defining the structure–activity relationships of lipid biological targets.

1. Introduction

A recent commentary described chemical biology as best performed via partnerships to allow cutting edge chemistry to shed light on otherwise impenetrable biological questions (Ostler, 2007). Although much of Robert (Bob) Bittman's career predated recognition of chemical biology as an interdisciplinary research field, Bob's contributions to science can now clearly be identified as chemical biology. Bob and others have been instrumental in providing synthetic lipids and bioactive lipid analogs that have been valuable tools to elucidate the types of molecular interactions and assemblies formed in vivo by lipids, either with other lipids or with proteins. A small set of synthetic lipid examples from the sterol, sphingolipid, and glycerolipid classes and the lipid assemblies these chemical tools have been utilized to understand are reviewed here.

2. Sterols: fluorescent cholesterol analogs shed light on membrane microdomains and protein interactions with cholesterol

Cholesterol serves a critical role in the biophysics of cell membranes, as a precursor for all steroid hormones and bile acids, as well as serving as a signaling molecule (Cortes et al., 2014). Analogs of bioactive lipids, including cholesterol, with either fluorescent or fluorescence-quenching groups have proven tremendously useful tools for studies of the biophysical properties of membranes (Bagatolli and Needham, 2014), the identification of specific lipid–protein binding interactions (Schwarzmann et al., 2014), investigation of lipid–protein colocalization in cell membranes (Schwarzmann et al., 2014), protein and peptide membrane penetration, and in characterizing membrane microdomains (Schwarzmann et al., 2014; Takatori et al., 2014; Klymchenko and Kreder, 2014; DeWitt and Dunn, 2015). In 2006, Bob Bittman's research group prepared and characterized fluorescent analogs of cholesterol and coprostanol to address limitations of other analogs available at the time (Li et al., 2006). In particular, intrinsically-fluorescent cholesterol analogs, cholestatrienol and dehydroergosterol, suffered from poor quantum yields and excitation wavelengths that overlapped with absorbances of many types of biomolecules (Li et al., 2006; Gimpl and Gehrig-Burger, 2011). Additionally, synthetic cholesterol analogs prepared with nitrobenzooxadiazole (NBD) and dansyl groups at varying positions on

Abbreviations: AGP, alkyl glycerol phosphate; BK, big conductance voltage-gated potassium channel; BODIPY, dipyrromethaneboron difluoride; CAPK, ceramide-activated protein kinase; CRAC, cholesterol recognition/interaction amino acid consensus; DHPE, 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; EC_{50}, effective concentration giving 50% activity; EDG, endothelial differentiation gene; Fn2, fibronectin type II; GPCR, G-protein coupled receptors; LPA, lysophosphatidic acid; LPA_1, lysophosphatidic acid receptor 1; MOE, molecular operating environment software; MOM, mitochondrial outer membrane; NBD, nitrobenzooxadiazole; NPC1, Niemann-Pick C1; PAF, platelet activating factor; PC, phosphocholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; SAR, structure–activity relationships; SM, sphingomyelin; TM, transmembrane.

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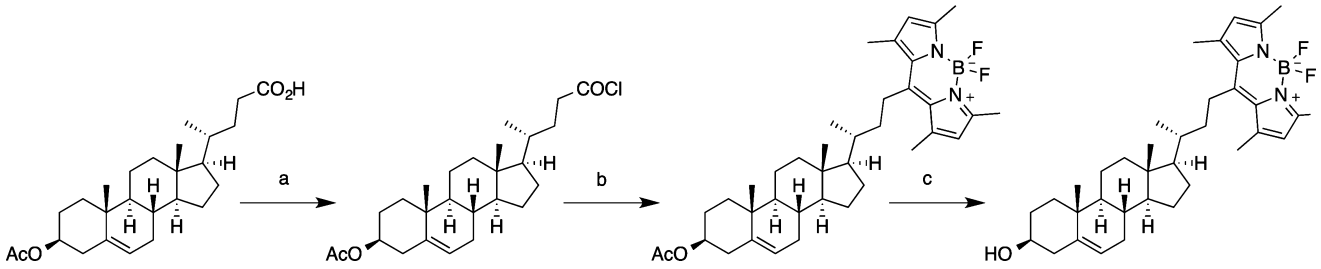

Scheme 1. Reagents and Conditions: (a) $(COCl)_2$, CH_2Cl_2, 0 °C to room temperature, overnight; (b) (i) 2,4-dimethylpyrrole, CH_2CH_2, reflux, 4 h, (ii) BF_3ŸOEt_2, triethylamine, rt, overnight; (c) K_2CO_3, methanol, rt, 2 days.

the steroid ring system differed from cholesterol with respect to partitioning in the ordered and disordered lipid phases in membranes (Li et al., 2006; Gimpl and Gehrig-Burger, 2011). Scheme 1 shows the synthetic approach used to prepare a cholesterol analog bearing a hydrophobic dipyrromethaneboron difluoride (BODIPY) fluorophore in the aliphatic chain (Li et al., 2006). The strategy utilizes an excess of dimethylpyrrole reacting with an acyl halide followed by treatment with boron trifluoride etherate to form BODIPY without residual hydrophilic functionality in the aliphatic chain.

The cholesterol analogs containing the BODIPY fluorophore were further characterized with regards to their lipid partitioning behavior. BODIPY-cholesterol showed ideal mixing behavior with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and supported lipid raft formation in multilamellar vesicles with sphingomyelin in a similar fashion as unmodified cholesterol (Li et al., 2006). The behavior of BODIPY-cholesterol in membranes was also investigated using molecular dynamics simulations, which demonstrated that BODIPY-cholesterol and cholesterol have similar impacts on ordering of nearby lipid chains and the BODIPY group prefers a location near the center of the membrane, where it exhibits limited perturbations on the membrane (Holtta-Vuori et al., 2008). Correlated measurement of fluorescence and toplogical features of planar supported bilayers using fluorescence-atomic force microscopy demonstrated that the partitioning of BODIPY-cholesterol was dependent on the bilayer composition, showing less domain-selective partitioning when sphingomyelin with an 18-carbon acyl chain was included than when sphingomyelin with a 16-carbon acyl chain was included (Shaw et al., 2006).

A range of biochemical studies demonstrated that BODIPY-cholesterol exhibited important biochemical similarities to cholesterol, including growth stimulation of sterol-auxotrophic cells, release from cells to ApoAI, and accumulation in sterol-enriched zebrafish tissues during development after microinjection into egg yolk sacs (Holtta-Vuori et al., 2008).

BODIPY-cholesterol has proven useful for imaging applications in live cells (Holtta-Vuori et al., 2008). The photostability of BODIPY-cholesterol is a critical property for time-lapse fluorescent imaging of cholesterol-labeled cellular organelles (Holtta-Vuori et al., 2008). Fig. 1 demonstrates the dynamic behavior of such organelles in wild type cells, in which over 10% of organelles showed movement at least 1.5 μm from the starting point over a 10 s time period. This dynamic behavior is strongly attenuated in M12 cells lacking functional Niemann-Pick C1 (NPC1) protein (Holtta-Vuori et al., 2008), which are characterized by cholesterol deposits in the late endosomal compartment and which have a known endosomal motility defect (Ko et al., 2001).

BODIPY-cholesterol has been used to investigate microdomains in lipid systems. Cholesterol partitioning in ternary lipid mixtures showed a striking dependence of the relative proportion of cholesterol in the mixture (DeWitt and Dunn, 2015). Fig. 2 compares fluorescent probe distribution in ternary lipid monolayers as a function of mole percent cholesterol, using either BODIPY-cholesterol or Texas Red DHPE (DHPE = 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt) probes. The Texas Red DHPE was included as a known marker of the lipid expanded phase, which in these mixtures would be rich in DOPC (DOPC = 1,2-dioleoyl-*sn*-glycero-3-phosphocholine). This figure shows that BODIPY-cholesterol partitions into the same lipid phase as Texas Red DHPE at low cholesterol concentration, with a reversal of lipid phase preference beginning at only 3 mole percent cholesterol and a complete reversal evident at 20 mol% cholesterol. This behavior is not dependent on the identity of the lipid forming the condensed phase as similar results are obtained using either DPPC (DPPC = 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) or sphingomyelin (SM).

BODIPY-cholesterol was used to characterize the impact of mammalian seminal plasma protein PDC-109 (also called BSP-A_1/A_2) on cholesterol dynamics in lipid bilayers (Scolari et al., 2010). PDC-109 contains a fibronectin type II (Fn2) domain, placing it in a family of proteins involved in altering the sperm cell plasma membrane composition during maturation through stimulation of

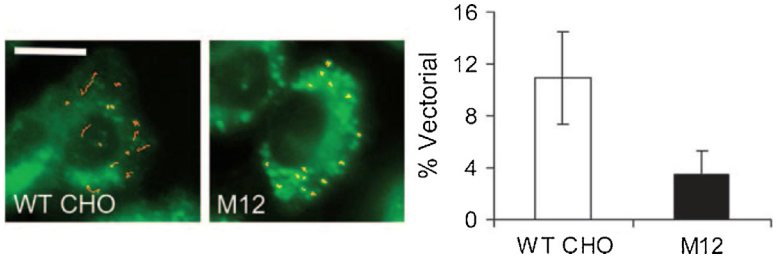

Fig. 1. Comparison of BODIPY-cholesterol labeled organelle dynamics in wild type and M12 cells lacking functional Niemann-Pick C1 protein. Cells were labeled for 2 min with cyclodextrin complexes of BODIPY-cholesterol and time-lapse images at 1 frame/second were acquired. Vectorial movement was based on organelles tracked over 10 frames in which movement to 1.5 μm from the starting point occurred.
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