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Tandem Facial Amphiphiles for Membrane Protein Stabilization

Pil Seok Chae,[†] Kamil Gotfryd,[‡] Jennifer Pacyna,[§] Larry J. W. Miercke,[||] Søren G. F. Rasmussen,[⊥] Rebecca A. Robbins,[||] Rohini R. Rana,[§] Claus J. Loland,[‡] Brian Kobilka,[*,⊥] Robert Stroud,[*,||] Bernadette Byrne,[*,§] Ulrik Gether,[*,‡] and Samuel H. Gellman[*,†]

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, United States, Department of Neuroscience and Pharmacology, The Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark, Department of Life Sciences, Imperial College London, London, SW7 2AZ, U.K., Department of Biochemistry and Biophysics, University of California, San Francisco, California 94158, United States, and Molecular and Cellular Physiology, Stanford University, Stanford, California 94305, United States

Received August 13, 2010; E-mail: gellman@chem.wisc.edu; gether@sund.ku.dk; b.byrne@imperial.ac.uk; stroud@msg.ucsf.edu; kobilka@stanford.edu

Abstract: We describe a new type of synthetic amphiphile that is intended to support biochemical characterization of intrinsic membrane proteins. Members of this new family displayed favorable behavior with four of five membrane proteins tested, and these amphiphiles formed relatively small micelles.

Membrane proteins (MPs) play crucial roles in biology, but these proteins are difficult to handle and analyze because of their physical properties.[1] The native conformations of MPs display extensive nonpolar surfaces, which is necessary for residence in a lipid bilayer but leads to denaturation and/or aggregation in an aqueous medium. Detergents, such as dodecyl-β-D-maltoside (DDM), are typically employed to render MPs soluble by coating nonpolar protein surfaces.[2] However, not all MPs can be maintained in native-like conformations when solubilized with conventional detergents.[3] Moreover, even when a native conformation can be achieved, the MP−detergent complex may manifest unfavorable properties with regard to structural analysis (inability to crystallize and/or too large for NMR). Since our understanding of membrane protein structure and function remains poorly developed relative to understanding of soluble proteins, there is a persistent need for new amphiphilic "assistants" that can promote solubilization and manipulation of MPs.[4]

Several groups have reported creative implementations of the "facial amphiphile" concept for the design of novel agents that display favorable behavior with selected membrane proteins.[5] McGregor et al., for example, have reported lipopeptides that are intended to match the width of a lipid bilayer and to form a sheath around nonpolar surfaces of MPs.[5c] Zhang et al. have developed cholate-based amphiphiles in which hydrophilic maltose units project from one side of the rigid and hydrophobic steroidal skeleton.[5d] Here we disclose the design of "tandem facial amphiphiles" (TFAs), which contain a pair of maltose-functionalized deoxycholate units. Unlike previous cholate-based designs, the TFAs are long enough to match bilayer width,[6] and unlike lipopeptides, the TFAs are readily synthesized in large quantities. We show that one TFA forms micelles containing only six molecules and that simple TFAs can be used to maintain a variety of MPs in native-like states in aqueous solution.

[†] University of Wisconsin.
[‡] University of Copenhagen.
[§] Imperial College London.
[||] University of California.
[⊥] Stanford University.

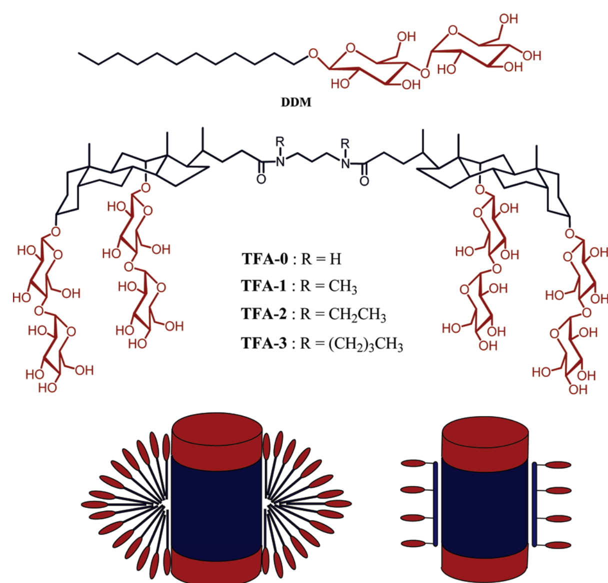

Figure 1. Chemical structures of DDM (*top*), tandem facial amphiphiles (TFAs, *middle*), and schematic representation of membrane proteins interacting with DDM (*bottom left*) and TFAs (*bottom right*).

A set of four TFAs was generated from a deoxycholate-bis-maltoside building block via linkage with a diaminopropane unit (Figure 1). Molecular mechanics calculations suggest that an extended conformation of the TFA backbone has a length that is comparable to the width of a typical lipid bilayer (~30 Å).[6] These TFAs vary in the appendage on the amide nitrogen atoms. Each amphiphile could be obtained in excellent purity (>98%) and good overall yield (~65%) in five straightforward synthetic steps with two chromatographic purifications.[6] Multigram quantities are readily available.

The TFAs displayed interesting behavior in water. TFA-0 forms a hydrogel at concentrations >0.4 wt %, and this compound was not studied further. The other three TFAs are soluble to 5−10 wt % in aqueous media. Critical micelle concentrations (CMCs) were determined by monitoring solubilization of a hydrophobic fluorescent dye, dicyclohexatriene,[7] and the hydrodynamic radii (R_h) of the micelles were determined via dynamic light scattering (DLS).[6] Table 1 compares the data for TFAs with those for DDM, a conventional detergent that is very widely used for MP applications; DDM and our TFAs share maltose as their hydrophilic moieties. CMC values of the three TFAs are smaller than that of DDM, whether the CMC is measured in units of mM or wt %. The micelles

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