Facial Amphiphiles

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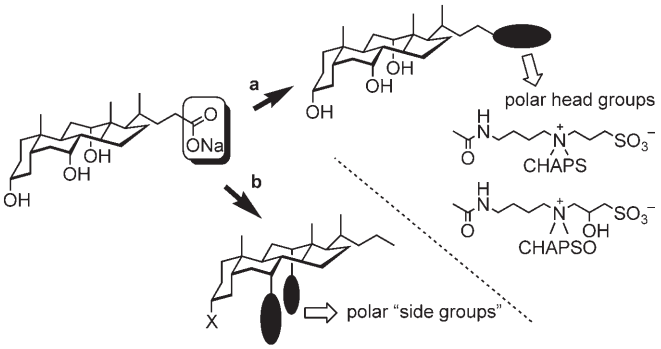
Designing Facial Amphiphiles for the Stabilization of Integral Membrane Proteins**

Qinghai Zhang, Xingquan Ma, Andrew Ward, Wen-Xu Hong, Veli-Pekka Jaakola, Raymond C. Stevens, M. G. Finn, and Geoffrey Chang*

Integral membrane proteins (IMPs) are essential for cell function and comprise more than 50% of human drug targets. However, they are notoriously difficult to handle because they are unstable and may lose function outside the lipid bilayer. Detergents, structurally similar to cellular lipids with an alkyl chain at one end and a polar head group at the other, are indispensable in the solubilization and purification of IMPs.[1] However, IMPs still tend to denature or aggregate in the presence of common detergents, which presents a significant challenge for the handling of proteins for biochemical assays and structural analysis. The development of new types of amphiphilic molecules that can stabilize IMPs is therefore of great value for both functional and structural investigation.[2]

Departing from the canonical "polar-head/nonpolar-tail" design of detergents, we have turned to molecules that exhibit facial amphiphilicity; an example is cholate, which projects three hydroxy groups in the same direction (Scheme 1). Cholate has a long history in membrane biochemistry, but its use is restricted to pH > 7.5 owing to the necessary ionization of the pendant carboxylic acid. Replacement of the carboxylate group of cholate with other polar head groups to give, for example, the zwitterionic CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) and CHAPSO (3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate; Scheme 1, path a) extends the utility of the cholate skeleton.[3] These structures are mostly used in protein reconstitution owing to their high critical micelle concentrations (CMCs).[4] In spite of more than a decade of effort, such steroid-based amphiphiles have yet to replace traditional detergents in the crystallization of an IMP for X-ray analysis.



Scheme 1. Amphiphile designs derived from cholic acid; through pathway a: traditional "polar-head/nonpolar-tail" structures; through pathway b: our proposed facial motif. X = H, OH, or other polar groups.

The three hydroxy groups in cholate and its derivatives provide only a small degree of facial amphiphilicity, leaving the very polar carboxylate or the zwitterionic end unit to provide the main driving force for aqueous solubility in the same manner as in classical detergents. Herein we introduce a different design derived from cholic acid, and demonstrate that a molecule based on this design is able to maintain the stability of two IMPs that we have tested so far.

We have chosen to deviate from the structure of most other steroid-based facial amphiphiles in two ways (Scheme 1, path b). First, the terminal carboxylate of cholate was removed, leaving a short alkyl chain mimic to the structure of cholesterol. Second, uncharged polar groups were attached to the parallel hydroxy groups in the center of the cholic acid skeleton. In this way, the amphiphiles should be forced to pack face-to-face when self-assembling.

After surveying a number of polar "side groups," the facial amphiphile **1** was prepared with a D-maltoside unit attached to each of the 7α- and 12α-hydroxy groups of the cholate skeleton. A convenient synthesis was developed by direct glycosylation of readily available dihydroxy cholane (prepared in 70% yield from cholic acid in three steps without column purification) as outlined in Scheme 2. Compound **1** was found to be highly soluble and have a relatively low CMC of 0.01% (0.1 mM) in water at 25°C (Supporting Information), quite close to that of dodecyl-β-D-maltoside (DDM, 0.0087%, 0.17 mM), which is one of the most useful detergents for membrane protein purification and crystallization. The

[*] Prof. Q. Zhang, Dr. X. Ma, Dr. W.-X. Hong, Dr. V.-P. Jaakola, Prof. R. C. Stevens, Prof. G. Chang
Department of Molecular Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-9985
E-mail: qinghai@scripps.edu
A. Ward
Department of Cell Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Prof. M. G. Finn
Department of Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

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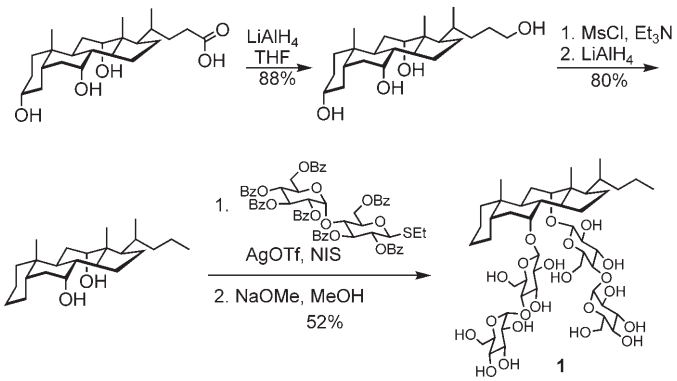
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 2. Synthesis of di-β-D-maltoside cholane **1**. NIS = *N*-iodosuccinimide.

CMC values of sodium cholate (0.41–0.60%, 9.5–14 mM), CHAPS (0.49%, 8 mM), and CHAPSO (0.50%, 8 mM) are 80–140 times greater than those of **1**, showing that the newly designed facial amphiphile has a much greater tendency to self-assemble. The hydrodynamic radius of the micelle formed by compound **1**, measured at a concentration of 0.03%, is 3.0–3.1 nm, slightly smaller than that of DDM (3.3–3.4 nm at the same concentration).

Compound **1** was first evaluated for solubilization and stabilization of MsbA from *S. typhimurium* (ST-MsbA), an ATP-binding cassette transporter protein composed of two transmembrane domains and two nucleotide-binding domains.[5] The purified protein in the candidate detergent was assayed for activity by a standard linked enzyme ATPase assay[6] to probe the protein stability. Undecyl-β-D-maltoside (β-UDM)—the detergent used for ST-MsbA purification and crystallization, conferring the highest protein activity in our previous experiments—was used as a positive control.

Other steroid-based detergents including sodium cholate, CHAPS, and CHAPSO were found to be very poor in solubilizing MsbA (lower than 15%). In contrast, compound **1** gave equivalently complete solubilization and initial ATPase activity ($1.21 \pm 0.06\ \mu mol_{ATP}\,min^{-1}\,mg^{-1}$) as β-UDM ($1.19 \pm 0.03\ \mu mol_{ATP}\,min^{-1}\,mg^{-1}$) under the same conditions (Figure 1a,b). When the enzymatic activity of the purified protein in β-UDM and compound **1** was followed for more than a week at room temperature, the protein solution in the presence of **1** remained clear and equally active, whereas in the presence of β-UDM, MsbA collapsed to form visible aggregates and lost more than 70% of its ATPase activity in two days (Figure 1c). While it is the soluble nucleotide-binding domain (NBD) of MsbA that is responsible for ATP hydrolysis, this domain alone showed no ATPase activity (Figure 1d), suggesting that the presence of a correctly folded transmembrane domain is necessary for full catalytic function.

Compound **1** was also investigated for the stabilization of bacteriorhodopsin (BR), isolated from the purple membrane of *Halobacterium halobium*. BR has a characteristic absorption at visible wavelength ($\lambda_{max} \approx 550$ nm in detergents) owing to the bound retinal ligand in the interior of the transmembrane region, serving as an excellent system for the testing of designed amphiphiles.[2a,d–g] BR was stored in the

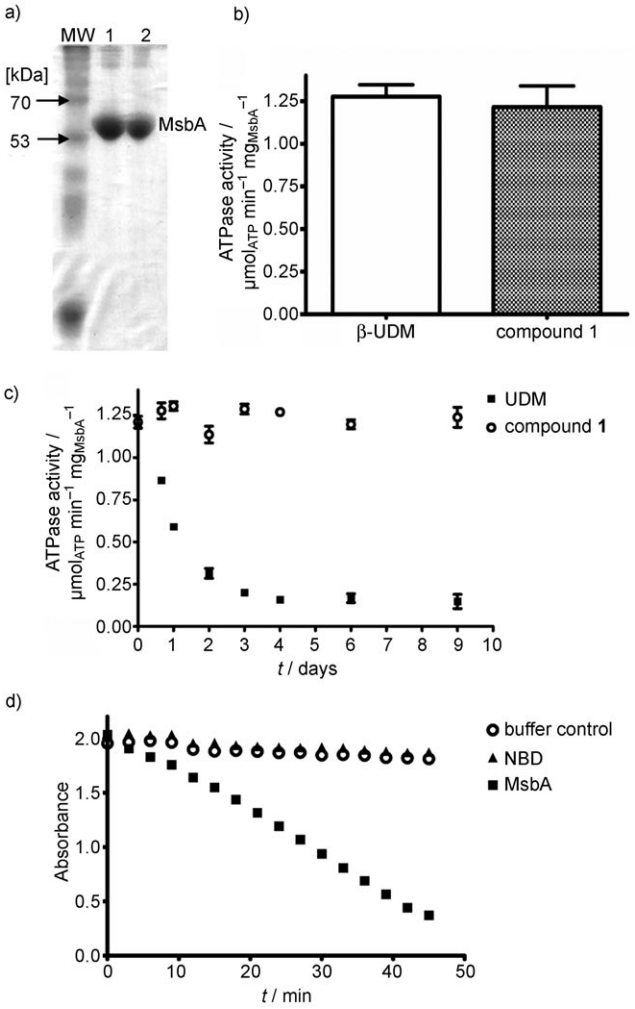


Figure 1. a) Sodium dodecylsulfate/polyacrylamide gel electrophoresis (SDS-PAGE) showing purity of MsbA extracted from membranes and purified with β-UDM (lane 1) and compound **1** (lane 2). b) ATPase activity of freshly purified MsbA in β-UDM and compound **1**. c) MsbA stability in the presence of β-UDM (solid square) and compound **1** (open circle) at room temperature, examined by the ATPase activity as a function of time. The data is the average of three measurements with standard error bars shown. d) ATP hydrolysis by NBD (solid triangle) and MsbA (solid square, in compound **1**), indicated by the decrease in absorbance of NADH at 340 nm.

presence of compound **1** for more than five months (pH 5.6, room temperature) and remained completely stable. In contrast, BR was gradually denatured in the presence of octyl-β-D-glucoside (OG), which has been previously used for BR crystallization and consequently as a standard for stability comparisons of the protein (Figure 2).[2d]

Classical detergents form large protein–detergent complexes, with micellar or prolate monolayer ring arrangements as proposed in the literature.[4] The structurally unique facial amphiphiles may have binding properties distinct from the classical detergents in segregating the hydrophobic transmembrane surfaces of IMPs. Compound **1** ($C_{48}H_{82}O_{22}$) has about twice the number of sugar and hydrophobic carbon atoms as DDM ($C_{24}H_{46}O_{11}$), presented a flat and a much larger hydrophobic surface similar to that of cholesterol. The

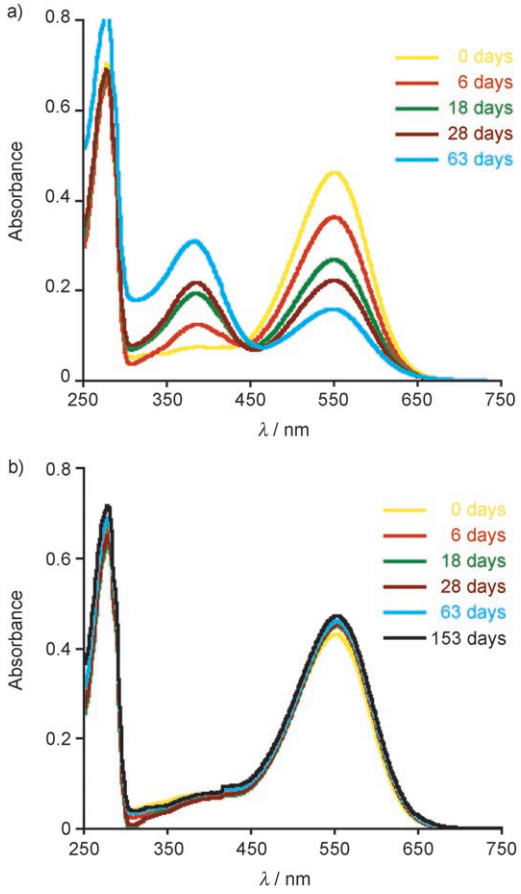


Figure 2. Absorbance spectrum of BR (pH 5.6, room temperature) in a) β-OG and b) compound **1**.

calculated length of the hydrophobic part of **1** is 13.4 Å (by Chem3D, MM2 energy minimization), and therefore two molecules of **1** can bridge most of the 30-Å hydrophobic dimension of a lipid bilayer. Using a colorimetric assay, we determined the maltoside detergent content in MsbA fractions eluted from well-equilibrated chromatography (Supporting Information).[7] The concentration of detergents associated with MsbA was obtained by subtracting the baseline detergent concentration from the measured overall detergent concentration. The detergent binding ratio was determined to be 219 ± 13 and 37 ± 4 mol/mol$_{MsbA}$ for β-UDM and compound **1**, respectively. The much smaller number of facial amphiphiles **1** bound to MsbA suggests that a smaller protein–detergent complex might be formed, and is consistent with the expectation that the larger hydrophobic surface area of the facial amphiphile gives rise to a more efficient stabilizing interaction with the membrane-spanning region of the protein.

The decarboxylated cholic acid platform provides a versatile and easily accessible platform for generating a new family of detergents useful for the stabilization of IMPs. Many other polar groups besides the glycosides described here could be attached to the α-hydoxy groups of the cholate skeleton, possibly providing the facial amphiphiles with different properties. The synthesis of other derivatives, and their use in the study of membrane-bound systems, are ongoing efforts in our laboratories.

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