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Mesitylene-cored glucoside amphiphiles (MGAs) for membrane protein studies: importance of alkyl chain density in detergent efficacy

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Dedication ((optional))

Abstract: Detergents serve as useful tools for membrane protein structural and functional studies. Their amphipathic nature allows detergents to associate with the hydrophobic regions of membrane proteins whilst maintaining the proteins in aqueous solution. However, widely used conventional detergents have major limitations in maintaining the structural integrity of membrane proteins and thus there are major efforts to develop novel agents with improved properties. We prepared mesitylene-cored glucoside amphiphiles (MGAs) with three alkyl chains and compared these agents with previously developed xylene-linked maltoside agents (XMASs) with two alkyl chains and a conventional detergent (DDM). When these agents were evaluated for four membrane proteins including a G protein-coupled receptor (GPCR), some agents such as MGA-C13 and MGA-C14 displayed markedly enhanced behaviours toward membrane protein stability relative to both DDM and the XMASs. This favourable behaviour is due likely to the increased hydrophobic density provided by the extra alkyl chain. Thus, this study not only describes new glucoside agents with potential for membrane protein research, but also introduces a new detergent design principle for future development.

Introduction

Membrane proteins play a variety of roles for proper cellular functions, including molecular transport, signal transduction and cell-to-cell communication. Malfunctions of membrane proteins are implicated in many diseases, reflected by the fact that more than half of all pharmaceuticals target these important molecules.^[1] Three dimensional structures of membrane proteins

are of major interest in drug discovery^[2], but only a few hundred novel membrane protein structures are currently available while there are more than 120,000 structures of soluble proteins.^[3] The slow progress in membrane protein structural study is strongly associated with their high propensity to aggregate and denature in an aqueous medium. Conventional detergents such as *n*-octyl- β -D-glucopyranoside (OG), *n*-dodecyl- β -D-maltopyranoside (DDM) and lauryldimethylamine-*N*-oxide (LDAO) are widely used to avoid such protein aggregation and denaturation.^[4] However, many membrane proteins surrounded even by these popular detergents tend to undergo structural degradation over time.^[5] This is particularly true for eukaryotic membrane proteins and membrane protein complexes. Thus, new detergents with enhanced properties are necessary to advance membrane protein science.^[6]

A number of non-conventional amphiphiles have been developed over the last two decades. Representatives include polymer-based nano-assemblies (amphiphilic polymers (amphipols)^[7a,b], nanodics (NDs)^[7c] and nanolipodisc particles^[7d] and peptide-based amphiphiles (e.g., lipopeptide detergents (LPDs)^[8a] and β -peptides (BPs)^[8b]). Small amphipathic molecules have also been invented as exemplified by tripod amphiphiles (TPAs)^[9a-c], facial amphiphiles (FAs)^[9d], glycodiosgenin (GDN)^[9e], neopentyl glycol (NG) class detergents (glucose neopentyl glycols (GNGs)^[9f,g], maltose neopentyl glycols (MNGs)^[9h-i], neopentyl glycol-derived triglucosides (NDTs)^[9k], and penta-saccharide detergents (PSEs)^[9l]. Of these small agents, NG class agents (GNGs and MNGs) have proved particularly interesting, as class members (e.g., MNG-3 and GNG-3) have contributed to the determination of more than 25 crystal structure membrane proteins. This clearly illustrates the important role of new amphiphiles in membrane protein research.^[10] In a recent study we described xylene-linked maltoside amphiphiles (XMASs)^[11] with a *p*-xylene linker that showed a favorable stabilizing effect on some membrane proteins, but were suboptimal for other membrane proteins such as the leucine transporter (LeuT) and the β_2 adrenergic receptor (β_2 AR) (Figure S1a). We hypothesize that the presence of two alkyl chains as the hydrophobic group in the XMASs is not ideal for tight packing of detergent alkyl chains around a target membrane protein, thereby limiting the protein stability conferred by these detergents in an aqueous environment. One strategy to address this issue is to enhance the hydrophobic density of the detergent molecules by increasing the number of alkyl chains. Thus, we developed three alkyl chains-bearing amphipathic agents, designated mesitylene-cored glucoside amphiphiles

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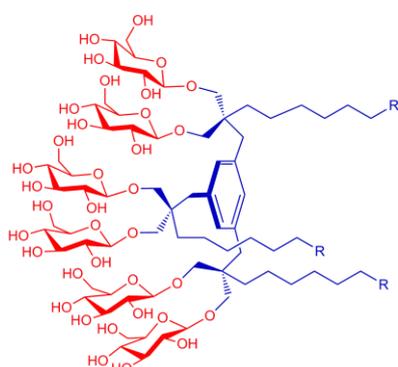
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(MGAs) which contain the mesitylene group as a linker. These agents bear one additional alkyl chain as well as one more branched diglucoside headgroup around the central benzene ring compared to the previously developed XMA^s.^[11] In the evaluation with four membrane protein systems, these new agents displayed markedly enhanced behaviors for all target proteins relative to the previously reported XMA^s^[11] and DDM, indicating a critical role of alkyl chain density in detergent behaviors toward membrane protein stability.

Results and Discussion

Detergent structures and physical characterizations



MGA-C10 : R = *n*-C₄H₉
MGA-C11 : R = *n*-C₅H₁₁
MGA-C12 : R = *n*-C₆H₁₃
MGA-C13 : R = *n*-C₇H₁₅
MGA-C14 : R = *n*-C₈H₁₇
MGA-C15 : R = *n*-C₉H₁₉

Scheme 1. Chemical structures of MGAs (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14 and MGA-C15) with alkyl chain length variation from C10 to C15. The number of carbons in the alkyl chain was utilized for detergent designation.

Initially, we prepared glucoside versions of the previously reported XMA^s, designated xylene-linked glucoside amphiphiles (XGAs) (Figure S1b), however the initial characterization identified a number of issues with these agents. First, XGA synthesis was much more difficult than that for the XMA^s, giving overall yields of less than 10%. The synthetic efficiency was significantly lower than that of the XMA^s (~60%), due to poor levels of glycosylation (~15%). Second, the XGAs showed unexpectedly low water-solubility, with the C7 alkyl chain XGA (XGA-C7) only marginally soluble in water (<1%). Thus, we could use only shorter alkyl chain agents (XGA-C4, XGA-C5 and XGA-C6) for evaluation with membrane proteins. In addition, the preliminary results of these evaluations indicated that the glucoside versions of XMA^s (i.e., XGAs) are unfavorable for membrane protein solubilisation and stabilization (see below). These agents tend to form large self-aggregates with diameters of 150 ~ 200 nm, significantly deviating from detergent micelles (Table S1 & Figure S2a), which is likely to be associated with their limited applicability for membrane protein manipulation. These unfavorable results prompted us to develop an additional class of glucoside amphiphiles with a mesitylene linker rather than *p*-xylene linker (MGAs; Scheme 1). Detergent alkyl chain length varied from C10 to C15 and this is incorporated into the detergent designation. Owing to the core structure change from

p-xylene to mesitylene, three alkyl chains and three branched diglucosides could be successfully implemented into the periphery of the central benzene ring. Each agent could be prepared without synthetic difficulty via a protocol comprising five synthetic steps, giving overall yields of ~25%. Interestingly, water-solubility was dramatically increased for the MGAs; the di-alkylated glucosides (XGAs) were poorly water-soluble with a C7 alkyl chain (<1%) while the tri-alkylated glucosides (MGAs) were more than 20% water-soluble even with a C15 alkyl chain. A similar trend was observed in a comparison with the di-alkylated maltoside versions (XMA^s); in this scaffold, a C12 alkyl chain was the maximum chain length for good water-solubility. Note that the XMA^s contain a higher number of glucose units than the MGA counterparts (8 vs 6). Thus, these results indicate that the MGAs possess a more water-soluble architecture than the XMA^s/XGAs, a feature likely contributing to their favorable properties for membrane protein manipulation.

Table 1. Molecular weights (MWs) and critical micelle concentrations (CMCs) of MGAs (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14 and MGA-C15) and a conventional detergent (DDM), and hydrodynamic radii (R_h ; $n = 5$) of their micelles.

Detergent	M_w [a]	CMC [μ M]	CMC [wt%]	R_h [nm] ^[b]
MGA-C10	1736.1	~8	~0.0014	2.9±0.01
MGA-C11	1778.2	~5	~0.009	3.0±0.04
MGA-C12	1820.3	~3.5	~0.0006	3.1±0.03
MGA-C13	1862.3	~3	~0.0006	3.2±0.03
MGA-C14	1904.4	~2	~0.0004	3.4±0.04
MGA-C15	1946.5	~1.5	~0.0003	3.6±0.02
DDM	510.1	~170	~0.0087	3.4±0.02

[a] Molecular weight of detergents. [b] Hydrodynamic radius of detergents measured at 1.0 wt% by dynamic light scattering.

Detergent micelles were characterized in terms of critical micelle concentrations (CMCs) and micelle size, both of which were estimated via fluorophore encapsulation using diphenylhexatriene (DPH)^[12] and dynamic light scattering (DLS), respectively. The results are summarized in Table 1. The CMC values of all MGAs (1.5–8.0 μ M) were significantly smaller than that of DDM (~170 μ M), indicating a strong tendency to form self-assemblies. Within the MGA series, the detergent CMC values decreased with alkyl chain length, giving MGA-C10 with the shortest alkyl chain the largest values (~8.0 μ M) and MGA-C15 with the longest alkyl chain the smallest value (~1.5 μ M). This is likely due to the increased hydrophobicity of the lipophilic groups with increasing alkyl chain length. Micelles formed by all the MGAs were smaller than or comparable to those formed by DDM (2.9–3.6 vs 3.4 nm). Detergent micelle size tends to increase with alkyl chain length in the architecture because of a gradual change in the geometry from conical to cylindrical shape. Yet, all of the MGAs prepared in this study self-assembled into

micellar structures with a diameter smaller than or comparable to DDM, mainly due to the presence of the large headgroup (i.e., three branched diglucosides). As can be seen in the DLS profiles, the micelles formed by these MGAs showed a single set of size populations with a narrow distribution, indicative of high homogeneity (Figure S2b).

Detergent evaluation with multiple membrane proteins

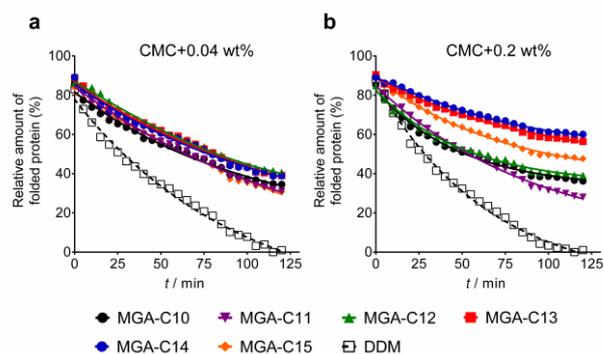


Figure 1. Thermal denaturation profile of UapA solubilized in a MGA (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15) and DDM used at two different concentrations: CMC+0.04 wt% (a) and CMC+0.2 wt% (b). The relative amount of folded protein was monitored as a function of time using the CPM assay carried out at 40°C for 120 min. The data is representative of two independent experiments.

In order to characterize the MGAs in terms of detergent efficacy for membrane protein stabilization, the new agents were first evaluated with a protein transporter, uric acid-xanthine/H⁺ symporter (UapA)^[13] from *Aspergillus nidulans*. The protein expressed in *Saccharomyces cerevisiae* was extracted by DDM from the membranes and then purified in 0.03% (w/v) of the same detergent. The DDM-purified UapA protein was diluted into buffer solutions containing individual MGAs or DDM to reach the final detergent concentration of the individual CMCs+0.04 wt%. After the dilution, the residual DDM concentration was 0.0002 wt%, far smaller than the CMC of DDM (0.0087 wt%). Protein thermostability was assessed over time using the dye, *N*-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM)^[14]. This non-fluorescent maleimide-containing molecule becomes highly fluorescent following specific conjugation with the sulfhydryl group of cysteine residues. Upon protein unfolding, a sulfhydryl group embedded in the protein interior tends to become exposed on the protein surface, leading to an increase in fluorescence intensity. Thus, the CPM assay provides a convenient means to estimate the change in the amounts of accessible sulfhydryl groups in a reaction medium, directly associated with the change in the relative amounts of unfolded target protein. As shown in Figure 1, all six MGAs were superior to DDM in terms of maintaining the folded structure of the protein at the individual CMCs+0.04 wt%. No noticeable differentiation was observed between the MGAs in this rather low detergent concentration. When detergent concentration was increased to the individual CMCs+0.2 wt%, however, efficacy

differences between the MGAs became more obvious. Of the MGAs, MGA-C13 and MGA-C14 were most effective followed by MGA-C15. All the other MGAs (MGA-C10, MGA-C11 and MGA-C12) having a rather short alkyl chain were less favorable in this regard although being still better than DDM. This result indicates the favorable architecture of the MGAs for preserving the folded state of the transporter.

The six MGAs were also evaluated with *Salmonella typhimurium* melibiose permease (MelB_{St})^[15]. *E. coli* membranes containing MelB_{St} were treated with 1.5 wt% individual MGAs or DDM and incubated for 90 min at 0°C for direct protein extraction. The amounts of soluble MelB_{St} after ultracentrifugation were determined by Western blot. The amount of soluble MelB_{St} in individual detergents was expressed as a percentage of total MelB_{St} in the untreated membrane sample (Figure 2). At 0°C, DDM showed high efficiency in the extraction of the permease while all MGAs were inferior to DDM, indicating the suboptimal behavior of MGAs. When the incubation temperature was increased to 45°C, however, the amount of soluble MelB_{St} significantly increased for all the MGAs; particularly, these with a long alkyl chain (MGA-C13, MGA-C14, or MGA-C15) maintain the soluble fractions to a level comparable to that with DDM. Detergent efficacy between DDM and the MGAs could be clearly discerned by a further temperature increase to 55°C. At this higher temperature, DDM yielded only a small amount of soluble MelB_{St} (<10%), implying that most DDM-solubilized permeases aggregated or denatured during the 90 min-incubation. In contrast, all the MGAs substantially retained soluble MelB_{St}, with the remarkable performance for MGA-C13 and MGA-C14 (90–100%) because no appreciable decrease in the amount of soluble MelB_{St} was detected. In the case of MGA-C15, the amount of soluble MelB_{St} was slightly decreased at 55°C. This result suggests that MGA-C13 and MGA-C14 have optimal structures for retaining MelB_{St} solubility. When the di-alkylated versions of MGAs (i.e., XGAs; XGA-C4, XGA-C5 and XGA-C6) were tested for this protein (Figure S3), all the XGAs were inferior to DDM with regard to extracting MelB_{St} from the membranes at 0°C and retaining the permease solubility at the elevated temperatures. These results clearly demonstrate that the tri-alkylated MGA architecture is superior to that of the di-alkylated XGAs at preserving MelB_{St} solubility/stability.

The favorable behaviors for UapA and MelB_{St} encouraged us to evaluate the MGAs with the leucine transporter (LeuT)^[16] from *Aquifex aeolicus*. MGA-C10 was inferior to the other MGAs in the evaluation with UapA and MelB_{St} and thus was not further evaluated. The transporter expressed in *E. coli* membranes was first extracted from the membranes using 1.0 wt% DDM and purified by the use of 0.05 wt% same detergent. The DDM-purified transporter was mixed with buffer solutions including individual MGAs or DDM to give a final detergent concentration of the individual CMCs+0.04 wt%. The residual DDM concentration was 0.005 wt%. The protein samples solubilized in the individual detergents were incubated for 12 days at room temperature and substrate binding activity of the transporter was measured at regular intervals *via* scintillation proximity assay

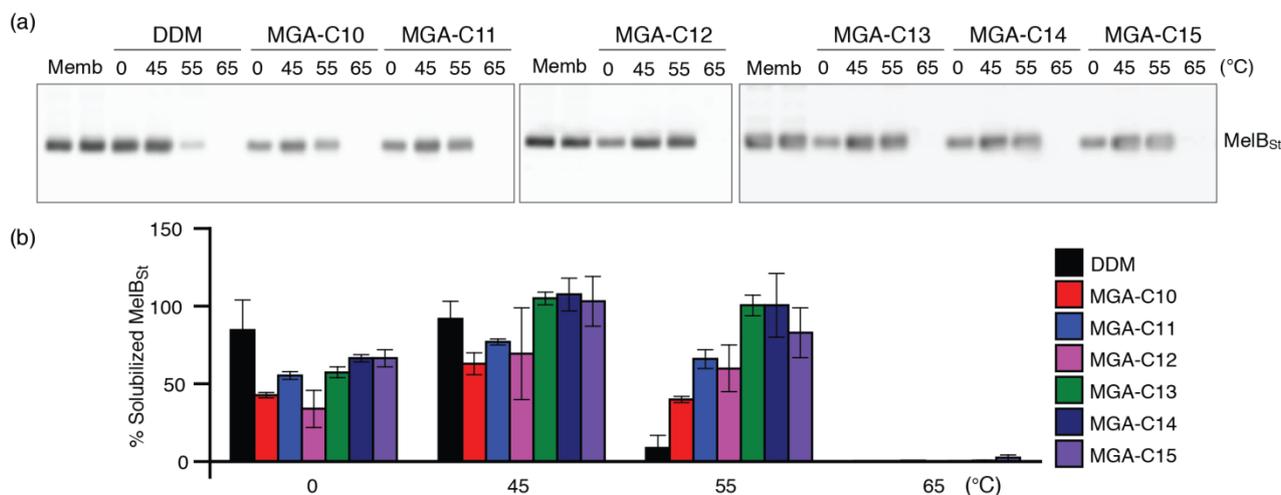


Figure 2. Thermostability of MelB_{St} solubilized in a MGA (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15) or DDM. The protein was extracted from *E. coli* membranes by treatment with 1.5 wt % individual detergents at four different temperatures (0, 45, 55, and 65 °C) for 90 min. The solubilized MelB_{St} was separated by ultracentrifugation and visualized by Western blot (a). The amounts of the soluble MelB_{St} were expressed as percentages of total MelB_{St} in the untreated membrane (Memb) and presented in histograms (b). Error bars, SEM, *n* = 3.

(SPA) using a radio-labelled substrate ($[^3\text{H}]\text{-Leu}$).^[17] As can be seen in Figure 3a, the DDM-solubilized transporter gave a gradual loss in substrate binding activity over time, resulting in ~40% retention of the initial activity after the 12-day incubation. All the MGAs were better than DDM in this context, with the best performance achieved for MGA-C11 followed by MGA-C12 and MGA-C13. The MGA-C11-solubilized transporter gave almost full retention in the substrate binding activity (~90%) after the 12-day incubation. Of the tested MGAs, MGA-C15 with the longest alkyl chain was the poorest at retaining transporter activity, but still slightly better than DDM. As detergent concentration was increased to the individual CMCs+0.2 wt%, the DDM-solubilized transporter more rapidly lost the substrate binding activity (Figure 3b). A similar trend was observed for the MGAs with a long alkyl chain (MGA-C14 and MGA-C15); these agents resulted in a significant decrease in the transporter activity (40–50% after 12 day incubation). In contrast, the MGAs with a short alkyl chain (MGA-C11, MGA-C12 and MGA-C13) were still very effective at preserving the activity of the transporter (~90% after the 12-day incubation). Thus, the outcome of the MGAs was dependent on detergent alkyl chain length. When the di-alkylated versions (XGAs; XGA-C4, XGA-C5 and XGA-C6) were evaluated with the transporter, none of these agents were better than DDM (Figure S4), consistent with the XGA results for MelB_{St}.

We next moved to the human β_2 adrenergic receptor ($\beta_2\text{AR}$), a G protein-coupled receptor (GPCR), for detergent evaluation.^[18] For this evaluation, the three MGAs (MGA-C12, MGA-C13 and MGA-C14) were selected as MGA-C11 and MGA-C15 showed the worst behavior for UapA and LeuT, respectively. The receptor was first expressed in Sf9 insect cells and then solubilized from the membranes using a conventional detergent (DDM). The receptor was further purified in 0.1 wt% DDM. The

DDM-purified receptor was diluted with individual MGAs- or DDM-containing buffers to give a final detergent concentration of the individual CMCs+0.2 wt%. After dilution, the residual DDM concentration in the MGA samples was 0.0007 wt%. The receptor stability was addressed by measuring the ligand binding activity using a radio-labelled antagonist ($[^3\text{H}]\text{-dihydroalprenolol}$, DHA).^[19] As a preliminary study, the activity of the receptor solubilized in the individual MGAs or DDM was measured after a 30 min dilution to allow detergent reconstitution. The MGAs (MGA-C12, MGA-C13 and MGA-C14) showed an initial receptor activity comparable to both each other and DDM (Fig. S5). However, a clear difference in detergent efficacy was observed between the MGAs and DDM when the receptor stability was monitored at regular intervals over a three-day incubation at room temperature (Fig. 3c). The DDM-solubilized receptor rapidly lost activity over time, resulting in only ~15 % retention of receptor activity at the end of the three-day incubation. A similar activity loss was observed for MGA-C12-solubilized receptor. In contrast, the MGA-C13 or MGA-C14-solubilized receptor lost ligand binding capacity much slower and additionally there was greater activity at the end of the incubation period (50-60%), with better performance for MGA-C14 relative to MGA-C13. In order to provide greater insights into the properties of the MGAs, DDM- or MGA-C13-solubilized $\beta_2\text{AR}$ was analyzed by size exclusion chromatography (SEC) following detergent exchange. MGA-C13 was chosen for this assessment as it showed effective stabilization of all four targeted membrane proteins. As can be seen in Fig. S6a, MGA-C13 formed smaller PDCs with the receptor than DDM, which may be favorable for membrane protein crystallization. Application of the DDM- or MGA-C13-solubilized $\beta_2\text{AR}$ to the SEC column in a detergent-free buffer revealed a significant efficacy difference between DDM and

MGA-C13; a large decrease in intensity of the monodisperse peak (~13 mL) and a concomitant appearance of the new broad aggregation peak (~10 mL) were observed for the DDM-solubilized receptor in detergent-free buffer (Fig. S6b). In contrast, only a slight change in the chromatogram was observed for the MGA-C13-solubilized receptor under the same condition. Thus, this MGA agent maintained receptor integrity even in a detergent-free buffer, presumably due to this detergent forming stronger interactions with the receptor than DDM.

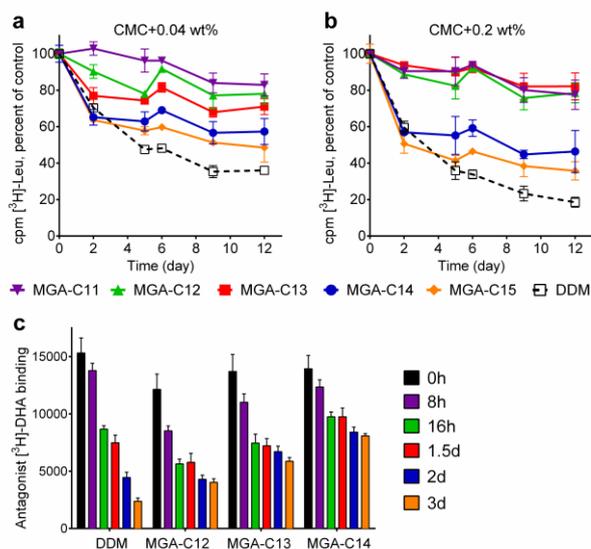


Figure 3. Long-term substrate or ligand binding activity for LeuT (a,b) and β_2 AR (c) solubilized in individual MGAs and DDM. Five MGAs (MGA-C11, MGA-C12, MGA-C13, MGA-C14 and MGA-C15) and three MGAs (MGA-C12, MGA-C13 and MGA-C14) were used for the evaluation with LeuT and β_2 AR, respectively. Two different detergent concentrations (a) CMC+0.04 wt% and (b) CMC+0.2 wt% were used for LeuT stability measurement while a single detergent concentration (CMC+0.2 wt%) was used for β_2 AR stability experiment. LeuT activity was measured at regular intervals over the course of a 12-day incubation at room temperature via scintillation proximity assay (SPA) using a radio-labelled substrate ($[^3\text{H}]$ -Leucine). β_2 AR ligand binding activity was assessed at regular intervals using the antagonist $[^3\text{H}]$ -dihydroalprenolol (DHA) over a 3-day incubation at room temperature. Error bars, SEM, $n = 3$.

In this study, we introduced mesitylene-cored glucosides (MGAs) with three alkyl chains and found that these agents were consistently more effective than DDM and di-alkylated versions (XGAs) for membrane protein stability. These agents were also clearly superior to the previously-reported di-alkylated maltosides (XMAs) at stabilizing membrane proteins, particularly for LeuT and β_2 AR. The previous study had shown that the XMAs were inferior to DDM for these membrane proteins.^[11] The well-behaved MGAs (MGA-C13 and MGA-C14) proved to be even superior to MNG-3, one of the most successful new amphiphiles^[5c,20], in terms of stabilizing UapA (Fig. S7). This finding appeared to be true for LeuT as well.^[9h,i] It is hard to narrow down which structural features are responsible for the enhanced efficacy of the MGAs. However, due to the presence of three alkyl chains around a central ring structure, the MGA molecules have increased alkyl chain density (i.e., hydrophobic

density) relative to the XGAs/XMAs with only two alkyl chains. Accordingly, the MGAs would generate a detergent micelle interior with increased hydrophobic density compared to the XGAs/XMAs. This may result in a stronger and thus more favorable detergent interaction with the protein hydrophobic surface, thus providing an optimal platform for effective protein encapsulation. The increased alkyl chain density also seemed to endow the MGAs with additional advantages. First, the MGAs were more water-soluble with increasing alkyl chain length than the equivalent XGAs and XMAs. Second, the MGAs formed relatively small micelles with a very low CMC value (1.5–8.0 μM), in stark contrast with the XGAs which formed large liposomes with a high critical aggregation value (~800 μM). Collectively, the current study revealed that the diverse detergent properties such as water-solubility, self-assembly morphology and, more importantly, membrane protein stabilization efficacy could be significantly improved by modulating the alkyl chain density (i.e., hydrophobic density) of detergent micelles. This detergent structure-property-efficacy relationship has not been studied in detail so far.

It is notable that the MGAs bear glucosides instead of maltosides as headgroups. In general, maltoside detergents (e.g., DDM) are more effective at stabilizing membrane proteins than glucoside agents (e.g., OG). However, glucoside agents are also widely used for membrane protein study, presumably as a result of the small headgroup (i.e., glucoside), which results in the formation of small PDCs. Small PDCs are often favorable for generation of high quality protein crystals and for nuclear magnetic resonance (NMR)-based protein structural study. Thus glucoside detergents have distinctive advantages over maltoside agents even if those agents have relatively limited ability to stabilize membrane proteins. A similar conclusion could be reached from the comparison of the GNGs with MNGs. For example, GNG-3 was generally inferior to the maltoside analog (MNG-3) in terms of maintaining membrane protein stability^[9f,h,i], but this agent has successfully facilitated the high resolution structure determinations of several membrane proteins.^[10o,p,21] This analysis (glucosides vs maltosides) implies that it is challenging to develop novel glucoside detergents with enhanced efficacy toward membrane protein stabilization relative to DDM, reflected by the fact that most novel detergents effective in this regard contain maltoside headgroups (e.g., MNGs, FAs, and GDN). Very recently, a class of glucosides called NDTs have attracted substantial attention as these glucosides were shown to be superior to DDM for multiple membrane proteins.^[9k] As a hydrophilic neopentyl glycol (NG) linker was used to connect the glucose group with the hydrophobic alkyl chain, however, the headgroup of this agent is an NG-glucose conjugate rather than glucoside alone. The current MGAs utilized a hydrophobic linker derived from dimethylmalonate to bridge the head and tail groups. Thus, these agents are the first examples of glucoside detergents conferring enhanced protein stability for the multiple membrane proteins relative to DDM.

Conclusions

Detergent efficacy tends to vary depending on the characteristics of target membrane proteins. Consequently, it is often the case that a detergent effective for one membrane protein is not favorable for another membrane protein. Thus, it is extremely important to evaluate a newly-developed agent for multiple membrane proteins to show general utility in membrane protein study. Furthermore, a detergent should be easily prepared for the wide spread in membrane protein society. Finding a detergent or detergent class with such general utility and high accessibility is extremely challenging because multiple favorable properties including good water-solubility, micelle formation, optimum hydrophile-lipophile balance (HLB) and small PDC formation need to be implemented within a single molecular architecture along with convenient synthesis. In the present study, a couple of MGAs (e.g., MGA-C13 and MGA-C14) showed general utility as these agents proved effective at stabilizing multiple membrane proteins. These results strongly indicate that these MGAs incorporate multiple favorable properties. Therefore, these agents will find use in future membrane protein studies and the design principles described here have the significant potential for the development and optimization of other new detergents.

Experimental Section

Experimental Details can be found in the Supporting information, including the synthesis and characterization of the MGAs, and membrane protein stability assay.

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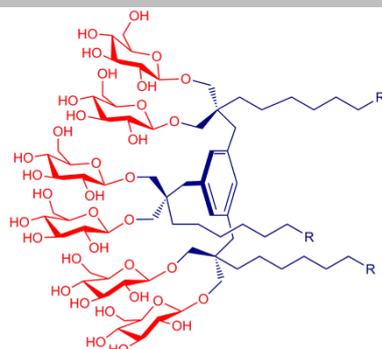
Keywords: amphiphile design • membrane proteins • detergents • protein stabilization • protein solubilisation

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FULL PAPER

Mesitylene-cored glucoside amphiphiles (MGAs) with three alkyl chains and six glucose units proved markedly superior to both a conventional detergent (DDM) and di-alkylated xylene-linked agents (XMAS) in terms of membrane protein stabilization. This study indicates that detergent alkyl chain density plays a crucial role in determining detergent efficacy.



Mesitylene-cored Glucoside Amphiphiles (MGAs)

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Mesitylene-cored glucoside amphiphiles (MGAs) for membrane protein studies: importance of alkyl chain density in detergent efficacy length