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Bolaamphiphile-Class Surfactants Can Stabilize and Support the Function of Solubilized Integral Membrane Proteins†

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Abstract

Bolaamphiphile-class surfactants composed of two hydrophilic (maltoside) headgroups connected by long saturated alkyl chains were tested for their ability to stabilize a solubilized membrane protein, *E. coli* diacylglycerol kinase (DAGK), and to sustain its native function. Members of this "Bis-MALT-$C_{18\text{-}28}$" series were poor solubilizers of DAGK in the absence of conventional detergent. However, mixed micelles of the bolaamphiphiles with either dodecylphosphocholine or β-n-decylmaltoside were more effective and enhanced DAGK's thermal stability relative to corresponding detergent-only conditions. Moreover, certain bolaamphiphiles were seen to be lipid-like by providing partial activation of DAGK's catalytic activity. Finally, addition of bolaamphiphiles to micellar NMR samples of DAGK did not result in a degradation of spectral quality, indicating their compatibility with high resolution structural studies. To our knowledge, this work represents the first documentation of the potential of bolaamphiphile-class surfactants for use in biochemical and biophysical studies of MPs.

The relative paucity of detailed biochemical and structural information regarding integral membrane proteins belies the tremendous importance of this class of proteins. One barrier to characterizing MPs is that they are usually less stable in the detergent micelles typically used to solubilize them than in native bilayers. A strategy used in nature to stabilize membrane proteins in extreme thermophiles is to employ membranes that are rich in "bolalipids"—lipids that have two polar headgroups that are separated on opposite sides of the membrane by linkage with complex hydrocarbon chains that span the entire membrane(1;2). Detergent-like compounds with a similar architecture, "bolaamphiphiles", have previously been synthesized and characterized(3-5) but have not, to our knowledge, been tested for possible use with membrane proteins. Here, we demonstrate the promise of bolaamphiphiles as a tool for working with solubilized membrane proteins.

Bis-β-maltosides with intervening n-alkyl chains ranging from 18 to 28 carbons were synthesized to generate the "Bis-MALT-$C_{18\text{-}28}$" series of bolaamphiphiles (Figure 1). Bis-MALT-C_{18} and -C_{22} exhibited reasonably high solubility in aqueous solutions at room temperature, but Bis-MALT-C_{24} and –C_{28} could only be solubilized if a conventional detergent is also present. Detergent-free solutions of Bis-MALT-C_{18} and –C_{22} were used in the final stages of purification of the helical integral membrane enzyme diacylglycerol kinase (DAGK).

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Supporting Information Paragraph

Full methodological details and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

As shown in Table S1, the yields of DAGK in these bolaamphiphiles were reduced relative to classical decylmaltoside (DM) micelles and the specific enzyme activity that could be recovered from the bolaamphiphile solutions was also lower. These results indicate that in detergent-free Bis-MALT-C_N solutions DAGK has much lower solubility than in conventional micelles and also tends to be irreversibly inactivated. We therefore sought to determine whether bolaamphiphile/detergent mixtures behaved more admirably.

All compounds of the Bis-MALT-C_N series were readily co-solubilized at a 2:1 wt:wt ratio of detergent (DM or DPC) to bolaamphiphile. These solutions were then employed in the final stages of DAGK purification. As shown in Table S1, purification yields of DAGK in Bis-MALT-C_N/detergent mixtures were reduced somewhat relative to yields when detergent-only solutions were used. However, the recovered specific enzyme activity of DAGK that was successfully purified and then assayed in a standard mixed micellar assay was comparable to the activities seen when DAGK was prepared in classical micelles (Table S1). Indeed, in mixed micelles of DPC or DM with Bis-MALT-C_{24} or –C_{28} the recovered activities matched or exceeded the purely micellar cases. The thermal stability of DAGK was then tested for each detergent and detergent-bolaamphiphile mixture. Time traces for activity loss at 70 °C are shown in Figure 2. For all bolaamphiphile-detergent mixtures except for Bis-MALT-C_{22}/DM, the $time_{1/2}$ for irreversible inactivation was extended—by as much as 19-fold in the case of Bis-MALT-C_{28}/DPC. For both Bis-MALT/DM and Bis-MALT/DPC mixtures, long chain ($C_{24\ or}\ C_{28}$) compounds were seen to yield the highest stability. However, for neither detergent was a consistent correlation of $time_{1/2}$ with bolaamphiphile chain length observed. This suggests that the structures and morphologies of the mixed bolaamphiphile/detergent assemblies may be complex in terms of dependency on bolaamphiphile chain length.

At standard assay substrate concentrations DAGK exhibits only very low catalytic activity in DPC or in DM micellesfootnote 2. It has long been known that DAGK requires the presence of lipid in micelles to be fully active (see review(6)). We examined whether or not compounds of the Bis-MALT-C_N series of bolaamphiphiles can play a lipid-like role in micelles to activate DAGK catalysis. As detailed in Table 1, Bis-MALT-C_{28} and, to a lesser degree, Bis-MALT-C_{24} both led to significant activation of DAGK. While the specific activity of the enzyme in the most favorable case—DM/Bis-MALT-C_{28}—remains well below the 100 U/mg activity of DAGK under ideal mixed micellar conditions, this result is promising, suggesting that even more favorable results may be anticipated if bolaamphiphiles are developed that have apolar-to-polar linkers and headgroups that more closely resemble the glycerophospholipids known to support the activity of DAGK under native conditions.

Previous structural studies of DAGK have been carried out primarily in DPC micelles using NMR methods(7)footnote 2. We have examined whether the presence of Bis-MALT bolaamphiphiles results in any loss of NMR spectral quality relative to detergent-only conditions. Shown in Figure 3 is the $^{1}H,^{15}N$-TROSY spectrum of DAGK in DPC/Bis-MALT-C_{24} both alone (top panel) and superimposed with the spectrum from DPC-only. While there are some modest spectral differences suggestive of subtle structural variation, there is high similarity of the two spectra, with no loss of quality resulting from the inclusion of Bis-MALT-C_{24}. Similar results were obtained in Bis-MALT-C_{28}/DPC mixtures (data not shown). Bolaamphiphiles therefore appear to be fully compatible with the sample conditions used for solution NMR and, most likely, X-ray crystallography-based structural biological studies of MPs.

[2]DAGK is actually active in DPC: its V_{max} in this detergent approaches that under optimal conditions. However, its substrate K_m are greatly elevated, such that the observed activity of DAGK in DPC micelles at the substrate concentrations used in this study (which are $>K_m$ for these substrates under optimal conditions) is very low.

An interesting question is whether Bis-MALT-C_N that have alkyl chains longer than C_{28} would behave even more favorably than the compounds tested. While this cannot be ruled out, we suggest it is unlikely. Bolaamphiphiles in the C_{24} to C_{28} range are expected to be able to fully span typical L_α-phase bilayers. Moreover, although Bis-MALT-C_{28} led to the highest degree of catalytic activation it was the C_{24} compound that led to the highest degree of protein stabilization when co-solubilized by DM.

What is the basis for stabilization of DAGK by bolaamphiphiles? We suggest that the interaction of membrane proteins with the mobile tails of detergent leads to destabilization of membrane proteins that stems from the entropic cost of dampening chain motion when tails bump up against the surface of the TMD of a well-ordered multispan MP. The motion of an extended bolaamphiphile chain in micelles or membranes is expected to be dramatically reduced relative to conventional detergents or conventional lipids, with the entropic cost for such immobility being “pre-paid” by anchoring the two headgroups on opposite ends of the assembly. If this model is correct then bolaamphiphiles are well-suited to preferentially interact with the TMDs of well-ordered folded MPs in a manner that does not require payment of an entropic penalty that destabilizes the MP. We therefore suggest that it is likely that the structure of a mixed detergent-bolaamphiphile-MP micelle will to some degree resemble MP protein complexes in classical bicelles(8) (Figure 4). This model will, of course, have to be tested through further characterization. However, the results of this first study of membrane protein/bolaamphiphile interactions may be promising enough to justify such studies and exploration of other classes of bolaamphiphiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DAGK	diacylglycerol kinase
DM	β-decylmaltoside
DPC	dodecylphosphocholine
MP	integral membrane protein
NMR	nuclear magnetic resonance

TM	transmembrane
TMD	transmembrane domain
TROSY	transverse relaxation-optimized spectroscopy

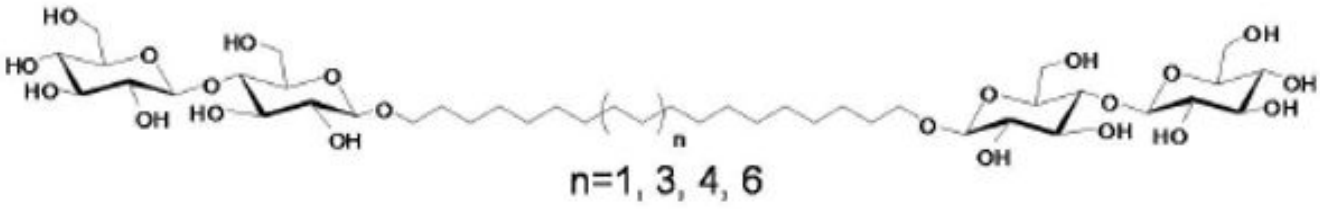

Figure 1.
Bolaamphiphiles used in this work: the Bis-MALT-C_N series, where N = 18, 22, 24, or 28.

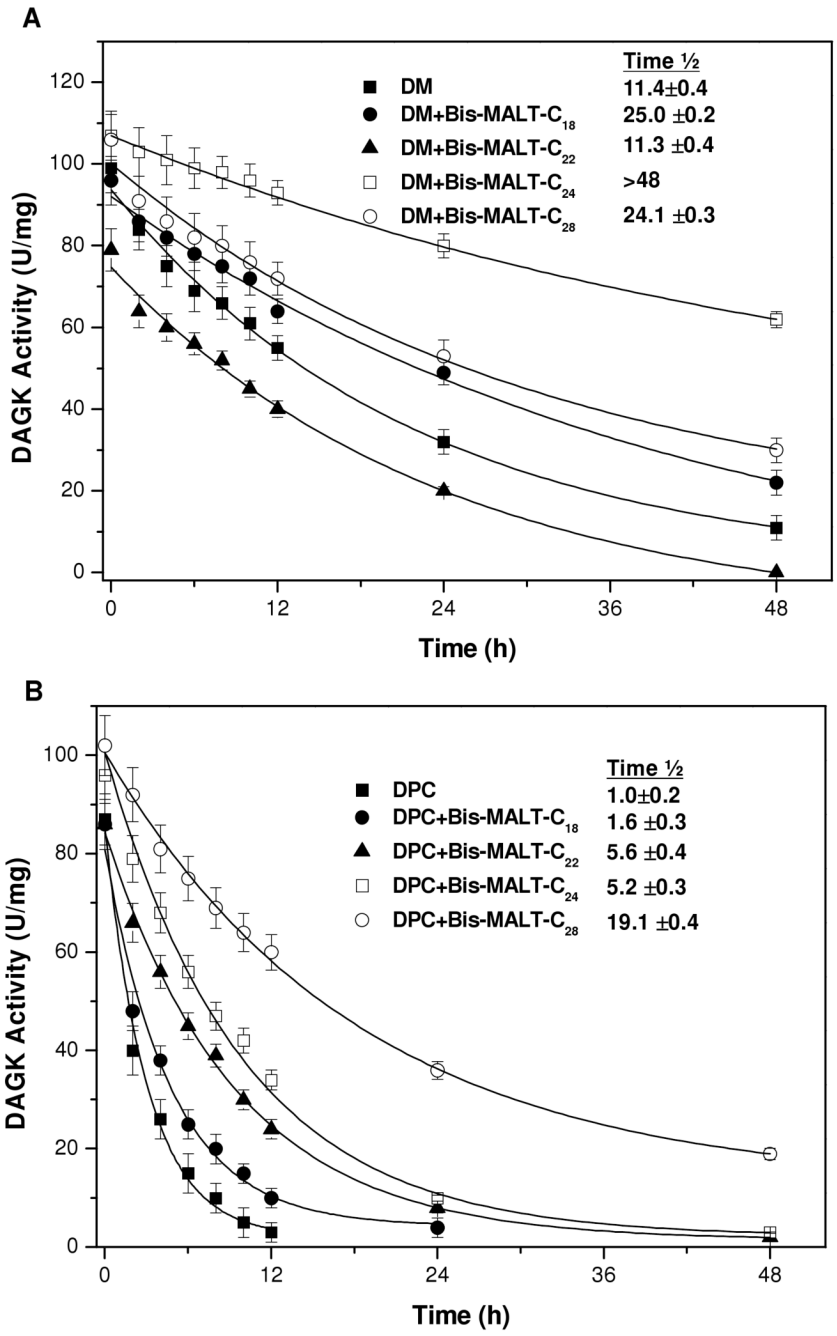

Figure 2.

Thermal stability of 0.2 mg/ml DAGK in 0.15%/0.3% bolaamphiphile-detergent mixtures at 70°C and pH 6.5. Aliquots from the 70°C samples were removed and subjected to DAGK activity measurement at 30 °C using the standard DM/cardiolipin mixed micellar assay system (see Supporting Information). Each time trace data set was fit by an exponential function to obtain the reported time$_{1/2}$ (hours) for irreversible inactivation. Units (U) of DAGK activity are micromoles of DAG converted to phosphatidic acid per minute at pH 6.5 and 30°C.

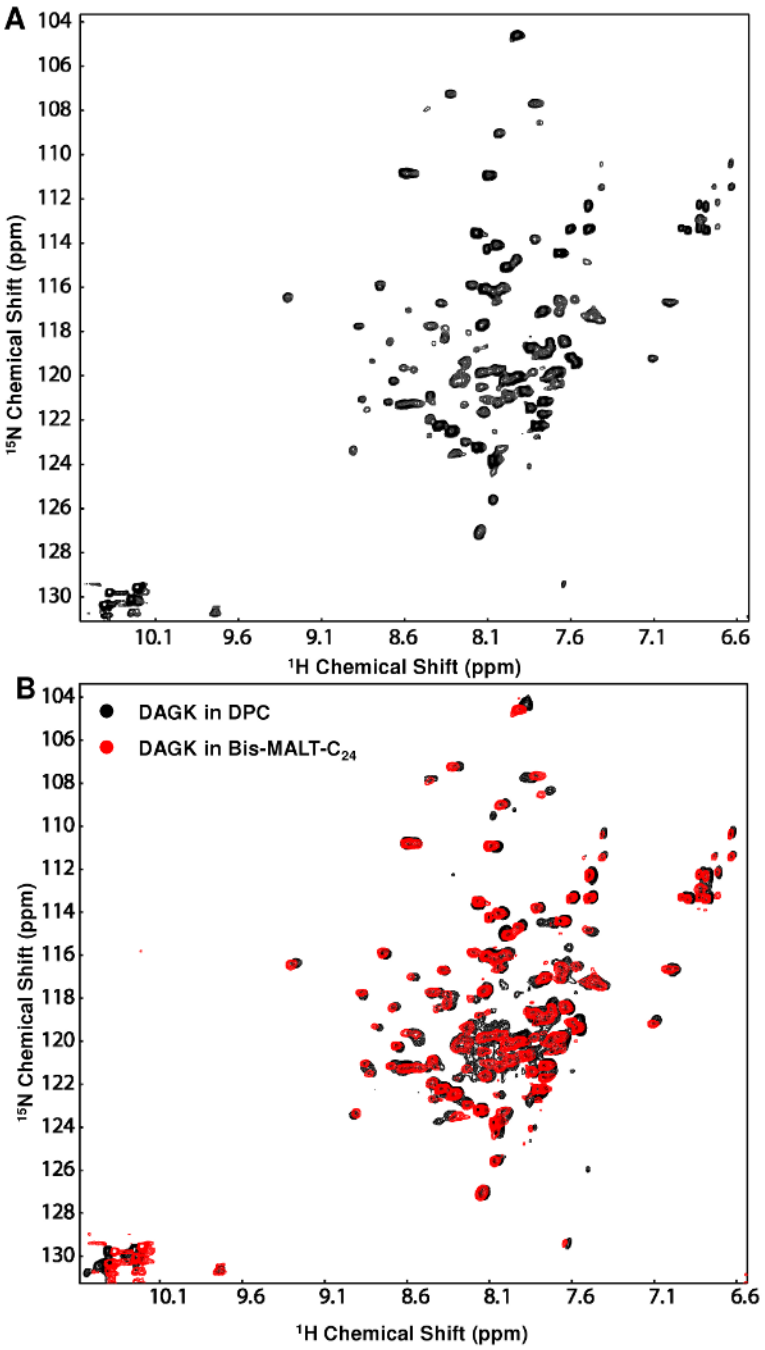

Figure 3.

Top panel: 800 MHz $^{1}H,^{15}N$-TROSY NMR spectrum of DAGK in a 6:1 (mol:mol) mixture of DPC and Bis-MALT-C_{24}, 45 °C and pH 6.5. **Bottom panel:** Superimposed 800 MHz spectra of DAGK in DPC/Bis-MALT-C_{24} (same as upper panel) and of DAGK in DPC micelles under otherwise identical conditions. Details of sample preparation and spectroscopy are given in the on-line supporting information.

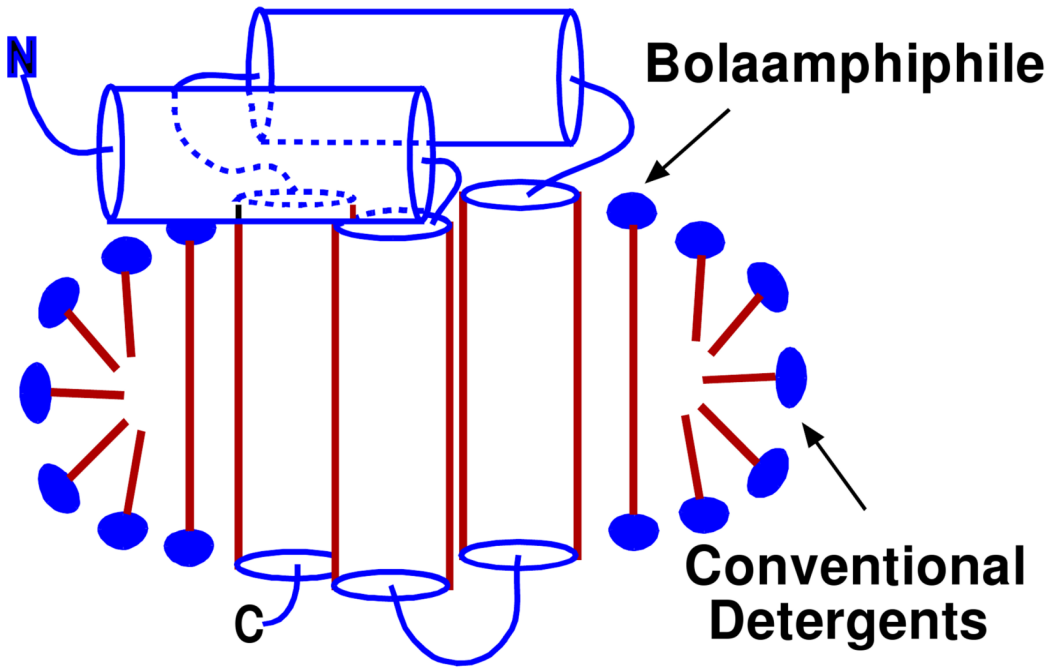

Figure 4.
Model for an integral membrane protein in bolaamphiphile-containing mixed micelles.

Table 1

Properties of Bolaamphiphile-Containing Solutions and of DAGK Solubilized Therein.

Detergents/Bolaamphiphile	Solubility in Buffer (no protein)[a]	Directly-Measured DAGK Activity[b]
Bis-MALT-C_{18}[c]	S	ND
Bis-MALT-C_{22}	S	ND
Bis-MALT-C_{24}	P	ND
Bis-MALT-C_{28}	P	ND
DM	S	0.37 ± 0.02
DM+ Bis-MALT-C_{18}	S	0.44 ± 0.02
DM+ Bis-MALT-C_{22}	S	0.49 ± 0.03
DM+ Bis-MALT-C_{24}	P/S	3.0 ± 0.2
DM+ Bis-MALT-C_{28}	P/S	12.6 ± 0.6
DPC	S	0.04 ± 0.01
DPC+ Bis-MALT-C_{18}	S	0.39 ± 0.02
DPC+ Bis-MALT-C_{22}	S	0.46 ± 0.02
DPC+ Bis-MALT-C_{24}	P/S	2.0 ± 0.1
DPC+ Bis-MALT-C_{28}	P/S	6.9 ± 0.4

[a] ND: not determined; S, soluble; C, cloudy; P, precipitation; P/S, soluble after heating at 100 °C.

[b] Specific activity (U/mg) measured in pH 6.5 assay mixtures at 30°C that contain the same bolaamphiphile and/or detergent-containing solution that DAGK was prepared in, rather than the cardiolipin/DM mixture normally used in the standard DAGK activity assay (in which DAGK exhibits activity of ca. 100 U/mg).

[c] Bolaamphiphile-only solutions contained 0.3% Bis-MALT-C_{18-28}, detergent-bolaamphiphile mixtures contained 0.3% detergent and 0.15% Bis-MALT-C_{18-28} (ca. 6:1 mole:mole ratio) and detergent-only solutions contained 0.5% DM or DPC.