Supporting Information for:

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

Qingxin Li[‡], Ritesh Mittal[§], Lijun Huang[§], Benjamin Travis[§], and Charles R. Sanders*[,‡]

[‡]*Department of Biochemistry and Center for Structural Biology, Vanderbilt University School of Medicine, Nashville, TN, 37232-8725, and* [§]*Anatrace-Affymetrix, Maumee, OH, 43537*

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Supporting Materials and Methods

Bolaamphiphiles and Detergents. Conventional detergents were purchased from Anatrace-Affymetrix. The "Bis-MALT-C_{18-28}" bolaamphiphiles used in this work, 1,N-bis-maltoside-C_N n-alkanes (N = 18, 22, 24, or 28), were synthesized and purified in-house using an efficient convergent synthetic route utilizing a common glycosyl donor intermediate. The glycosyl donor was synthesized in four steps with an overall yield of 70%. Sequentially, these steps proceed as a global protection of the maltose hydroxyl groups, bromination, and then hydrolysis of the anomeric center with a final conversion to the trichloroimidate, creating a suitable leaving group. These four steps were carried out with minimum requisite workup protocols while the resultant

donor trichloroimidate was separated by silica gel column chromatography with ethyl acetate in hexanes.

The various acceptor diols were synthesized using a two step protocol with an overall yield of 55-70% depending on their chain length. These two steps include a DIBAL-H reduction of the commercially available esters followed by Grubbs olefin metathesis reaction. The diol linkers were separated individually by silica gel column chromatography using a suitable ratio of EtOAc in hexanes.

The individual Lewis-acid catalyzed glycosylation reactions of the common trichloroimidate donor with the acceptor diol provided the respective protected target compound with a yield of approximately 80%. The respective β,β-isomer was separated at this stage by silica gel column chromatography using a suitable ratio of EtOAc in hexanes. Finally, the global deprotection of the hydroxyl protecting groups under basic medium and hydrogenation provided the target compounds in 60-75% yield range depending upon the chain length. The target compounds were purified by reverse phase C18 chromatography using a linear gradient of 50-100% methanol in deionized water.

Expression and Purification of DAGK from E.coli. DAGK was expressed and purified from *E.coli* (strain pSD005, a leucine auxotroph) as described(*1*). Briefly, a single colony of *E.coli* was inoculated into 4 ml of LB with antibiotics. The overnight culture was transferred into 1 L of M9 medium with antibiotics and leucine and incubated at room temperature with 240 rpm. When OD_{600} reached 0.8 the cells were induced with 1 mM IPTG and further incubated for 24 h. Cells were harvested by centrifugation at 12,000 g for 10 min and cell pellet was re-suspended into a buffer containing 75mM Tris-HCl pH 7.7, 0.3 M NaCl, 0.2 mM EDTA, and 10 mM β-hydroxyltoluene, and then gently agitated at 4 °C for about 1 h with 0.2 mg/ml lysozyme, 0.02 mg/ml DNase, 0.02 mg/ml RNase and 5 mM MgAcetate. Cells were then lysed by sonication on ice and extracted using the 3% Empigen (Fluka), a zwitterionic detergent. The cell lysate was

cleared by centrifugation at 20,000 g for 20 min at 4 °C. The supernatant was incubated with buffer-equilibrated Ni-NTA resin (Qiagen) for 2 hours. The resin was loaded onto a column and washed with a buffer containing 40 mM HEPES (pH 7.5), 300 mM NaCl, 10 μM β-hydroxyltoluene, 1.5% Empigen, 40mM imidazole (pH 7.8), at which point all impurities eluted from the resin. The resin was then re-equilibrated with a buffer (25 mM Na-PO_4, pH 7.2) that contains one of these four mixtures: (i) 0.5% beta-n-decylmaltoside (DM), (ii) 0.5% dodecylphosphocholine (DPC), (iii) 0.3% DM plus 0.15% Bis-MALT-C_N, or (iv) 0.3% DPC plus 0.15% Bis-MALT-C_N. DAGK was then eluted into the same detergent or detergent-bolaamphiphile mixture using a buffer that contains 250 mM imidazole, pH 7.8. The concentration of eluted DAGK was measured at 280 nm using an extinction coefficient of 2.1 OD units per mg/ml.

Preparation of Bolaamphiphile-Containing Solutions. Bis-MALT-C_N bolaamphiphiles were dissolved in buffer, which sometimes required heating the solution for 10 to 15 min, after which the bolaamphiphiles remained soluble even after cooling. Mixed micelles composed of the mixture of DPC or DM with a bolaamphiphile were prepared by mixing 10% detergent stock solutions with solid Bis-MALT-C_N, followed by repeated freeze-thawing using liquid nitrogen till the solution became clear.

DAGK Activity Assays. Catalysis of phosphorylation of diacylglycerol by DAGK was determined using an enzyme coupling system whereby the production of ADP by DAGK is coupled to the reactions of pyruvate kinase and lactic dehydrogenase leading to A_{340}-detectable oxidation of NADH(*2*). One unit of DAGK activity is equal to 1 μmol of diacylglycerol (DAG) converted into phosphatidic acid per minute at 30 ºC. The form of diacylglycerol used in this assay is *sn*-1,2-dihexanoylglycerol (DHG). The “standard mixed micellar assay” was used to measure the “recovered DAGK activity” where small aliquots of DAGK in bolamphiphile-containing solutions

were transferred into a standard assay mixture containing DM/cardiolipin mixed micelles as the model membrane(*2*). For this work we also carried out direct assays in which DM/CL was replaced with mixtures of DM or DPC with the Bis-MALT-C_N bolaamphiphiles (where N = 18, 22, 24, or 28). In these cases the DM or DPC concentration was 0.3% and the bolaamphiphile concentration was 0.15% (which corresponds to 15-18 mol%). It was observed that the pyruvate kinase/lactic dehydrogenase coupling enzymes used in this assay functioned normally in the presence of all bolaamphiphile-containing mixed micelles that were tested, at both 30 and 45 ºC. All activities measurements reported in this work represent the average of the results from three assays.

Determination of DAGK Thermal Stability. 50 µL aliquots of freshly purified 0.2 mg/ml DAGK solutions containing 250 mM imidazole, pH 6.5, 0.3% DM or DPC and 0.15% Bis-MALT-C_N were transferred into 0.2 mL PCR tubes and incubated at 70 °C. Samples were removed at time points and then stored at 25 °C until they were subjected to the standard mixed micellar assay at 30 °C. All measurements were carried out in triplicate. Recovered DAGK activity as a function of incubation time at 70 °C was plotted and then fit by a single exponential function using the program ORIGIN (http://www.originlab.com/) to determine the $time_{1/2}$ for activity loss in the detergent or detergent-bolaamphiphile mixtures of interest.

Collection of NMR Spectra for DAGK in Bolaamphiphile-Containing Solutions. DAGK was uniformly ^{15}N-labeled using M9 medium with $^{15}NH_4Cl$ and the labeled protein was purified into a solution containing 0.3 M imidazole, pH 7.8 plus either 0.5% DPC or 0.3% DPC plus 0.15% Bis-MALT-C_N. The pH of the eluted DAGK pool was adjusted to pH 6.5 and D_2O was added to 10%. The sample was then concentrated to 0.5 mM DAGK using a centrifugal ultrafiltration cartridge (10 kDa molecular weight cutoff, which also concentrates the micelles present). 2-D^{1}H-^{15}N-TROSY-HSQC(*3*) spectra were recorded at either 45 °C or 55 °C using either a Bruker 800 MHz

spectrometer equipped with a cryoprobe or a Bruker 600 MHz spectrometer equipped with a conventional TXI probe. The spectra were processed with NMRPipe(*4*) and visualized with NMRView(*5*).

Table S1. Properties of Bolaamphiphile-Containing Solutions and of DAGK Solubilized Therein.

Detergents/ Bolaamphiphile	Solubility in Buffer (no protein)[a]	Solubility of DAGK at 22 ºC[a]	Normalized Purification Yield of DAGK (mg/liter of culture)	Recovered Activity in DM/Cardiolipin Mixed Micelles	Directly-Measured DAGK Activity[b]
Bis-MALT-C_{18}[c]	S	S	2.3	84 ± 4	ND
Bis-MALT-C_{22}	S	C	5.3	71 ± 4	ND
Bis-MALT-C_{24}	P	ND	ND	ND	ND
Bis-MALT-C_{28}	P	ND	ND	ND	ND
DM	S	S	11.5	98 ± 5	0.37 ± 0.02
DM+ Bis-MALT-C_{18}	S	S	8.6	96 ± 4	0.44 ± 0.02
DM+ Bis-MALT-C_{22}	S	S	6.6	79 ± 4	0.49 ± 0.03
DM+ Bis-MALT-C_{24}	P/S	S	6.3	107 ± 7	3.0 ± 0.2
DM+ Bis-MALT-C_{28}	P/S	S	7.1	106 ± 6	12.6 ± 0.6
DPC	S	S	12.6	95 ± 5	0.04 ± 0.01
DPC+ Bis-MALT-C_{18}	S	S	8.0	86 ± 6	0.39 ± 0.02
DPC+ Bis-MALT-C_{22}	S	S	8.5	86 ± 4	0.46 ± 0.02
DPC+ Bis-MALT-C_{24}	P/S	S	9.6	96 ± 8	2.0 ± 0.1
DPC+ Bis-MALT-C_{28}	P/S	S	5.8	102 ± 10	6.9 ± 0.4

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

[b]Activity measured in assay mixtures that contain the same bolaamphiphile and/or detergent-containing solution that DAGK was prepared in, rather than the cardiolipin/DM mixture of the standard assay.

[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}, and detergent-only solutions contained 0.5% DM or DPC.

References for Supporting Text

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