Impact of Urea on Detergent Micelle Properties

Supporting Information

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Contents

1. Demicellization isotherms for cyclohexyl alkyl maltosides and alkyl glucosides

Figures S1 and S2 depict demicellization isotherms for cyclohexyl alkyl maltosides and alkyl glucosides, respectively, in the presence of 0–8 M urea.

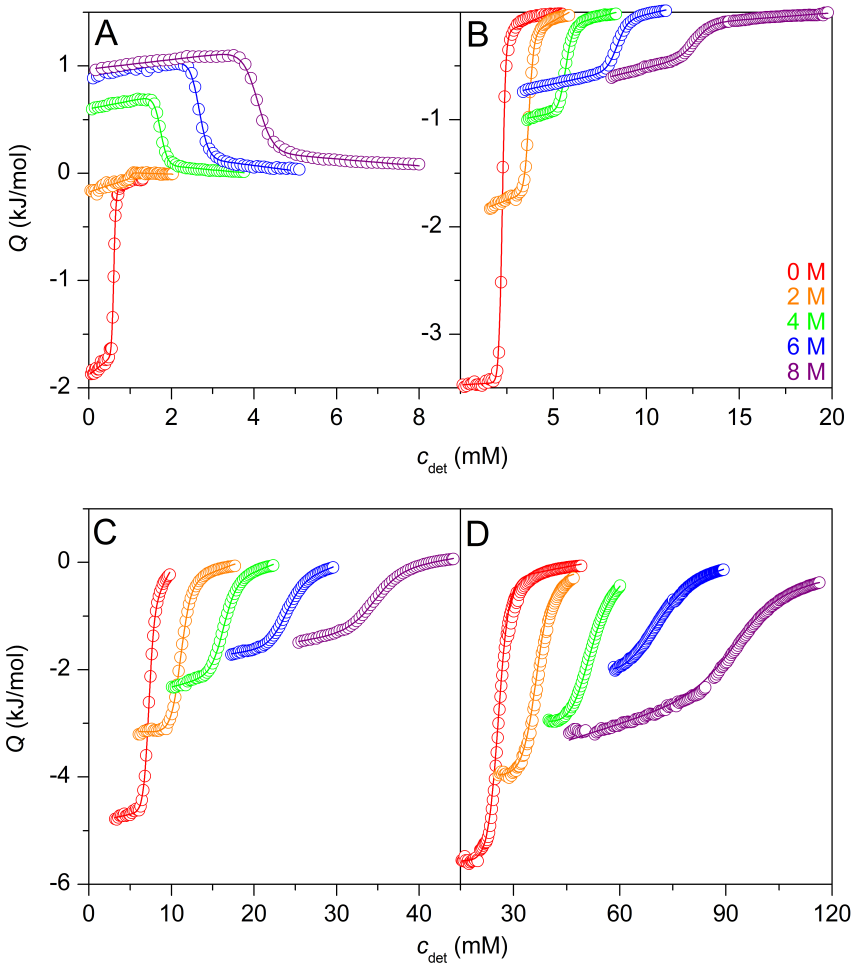

Figure S1. Demicellization of cyclohexyl alkyl maltosides at increasing urea concentrations studied by ITC at 25°C. (A–D) Normalized heats of reaction, Q, *versus* average detergent concentration in the cell during the injection, c_{det}, at urea concentrations of 0–8 M. Experimental data (*open circles*) and sigmoid fits (*solid lines*) according to Eq. 1 are shown for detergents having chain lengths of (A) 12, (B) 11, (C) 10, and (D) 9 C-atoms.

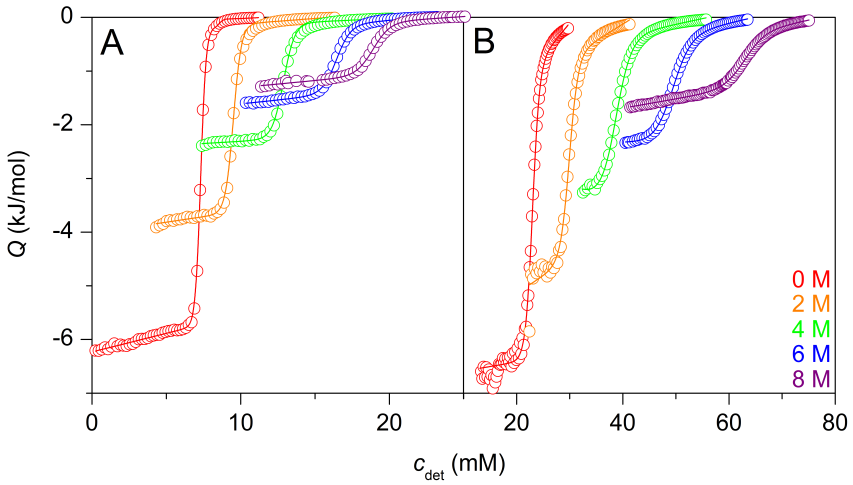

Figure S2. Demicellization of alkyl glucosides at increasing urea concentrations studied by ITC at 25°C. Normalized heats of reaction, Q, *versus* average detergent concentration in the cell during the injection, c_{det}, at urea concentrations of 0–8 M. Experimental data (*open circles*) and sigmoid fits (*solid lines*) according to Eq. 1 are shown for detergents having chain lengths of (A) 9 and (B) 8 C-atoms.

2. CMC and K^{mic} values in tabular form

Tables S1 and S2 provide all CMC and K^{mic} values, respectively, in tabular form.

Table S1. CMC values for alkyl maltosides, cyclohexyl alkyl maltosides (CyMals), and alkyl glucosides with chain lengths of 8–12 C-atoms.

CMC (mM)

c_{urea}	Maltosides					CyMals				Glucosides	
(M)	C8	C9	C10	C11	C12	C9	C10	C11	C12	C8	C9
0	21.6	6.85	2.03	0.588	0.150	25.7	7.25	2.25	0.607	23.0	7.30
2	30.9	9.89	3.07	0.928	0.256	36.4	11.1	3.69	1.03	29.9	9.51
4	41.3	14.0	4.54	1.45	0.453	50.5	16.3	5.66	1.73	38.6	12.9
6	53.9	19.8	6.67	2.20	0.658	67.8	23.8	8.43	2.67	49.3	16.4
8	75.2*	24.6	8.78	3.24	1.08	94.4	34.6	12.7	4.08	63.0	18.9**

*Estimate only because of very shallow demicellization isotherm (Figure 2F). **Value at 7 M urea because of NG precipitation at 8 M urea.

Table S2. Mole fraction partition coefficients, K^{mic}, for alkyl maltosides, cyclohexyl alkyl maltosides (CyMals), and alkyl glucosides with chain lengths of 8–12 C-atoms.

$K^{mic} \times 10^{-3}$

c_{urea}	Maltosides					CyMals				Glucosides	
(M)	C8	C9	C10	C11	C12	C9	C10	C11	C12	C8	C9
0	2.57	8.10	27.3	94.4	370	2.16	7.65	24.7	91.5	2.42	7.60
2	1.80	5.61	18.1	59.8	216	1.52	4.99	15.0	54.1	1.85	5.83
4	1.34	3.97	12.2	38.3	122	1.10	3.40	9.80	32.0	1.44	4.31
6	1.03	2.80	8.33	25.2	84.5	0.819	2.33	6.59	20.8	1.13	3.37
8	0.738*	2.26	6.32	17.1	51.6	0.588	1.60	4.37	13.6	0.882	2.93**

*Estimate only because of very shallow demicellization isotherm (Figure 2F). **Value at 7 M urea because of NG precipitation at 8 M urea.

3. Comparison of CMC values in buffer

All CMC values determined in the absence of urea in this study are in fair agreement with literature values obtained in water and simple salt solutions compiled in Table S3.

Table S3. Comparison of CMC values and mean aggregation numbers in water, simple salt solutions, or buffer for alkyl maltosides (M), cyclohexyl alkyl maltosides (C), and alkyl glucosides (G) with chain lengths of 8–12 C-atoms.

		CMC (mM)				agg. no.	
		this study	H_2O	NaCl[#]	buffer	H_2O	buffer
M	C8	21.6	23.3 (1)		19.5*,**; 25*** (2)	6*; 26 (3)	35–71[†††] (4)
	C9	6.9	6.0*; 7.6 (1)			25*	
	C10	2.0	1.8 (5); 2.1 (1)	1.8*	1.9[†] (6)	69*	82–90[†††] (7); 86–104[†††] (4)
	C11	0.59	0.59*; 0.64 (1)			71*	
	C12	0.15	0.17 (8); 0.17 (1)	0.12*	0.18[†] (6)	78–149 (8); 132 (9)	135–145[†††] (7); 135–155[†††] (4)
C	C9	25.7	34.5*	29.0*		5*	
	C10	7.3	7.6*	7.3*		25*	
	C11	2.3	2.4–5.0*	2.0*		47*	
	C12	0.61	0.56*			91*	
G	C8	23.0	18–20 (10); 23 (11,12)	23.4 (13)	21.8[†] (6); 29.6[††] (14)	27–100 (10)	80–100[†††] (7)
	C9	7.3	6.5*	6.0 (15)		133*	230–260[†††] (7)

[#], 0.1–0.15 M; *, Anatrace catalog; **, 20 mM HEPES; ***, 10 mM glycine (pH 10), 2 mM EDTA; [†], 10 mM HEPES (pH 7.2), 0.1 mM EDTA, 0.02% sodium azide; [††], 10 mM potassium phosphate (pH 7.1), 150 mM KF; [†††], 20 mM phosphate (pH 6.2), 150 mM NaCl.

4. Micellization thermodynamics and increments per methylene group in buffer

Figure S3 shows that $\Delta G^{\circ,\mathrm{mic}}$, $\Delta H^{\circ,\mathrm{mic}}$, and $-T\Delta S^{\circ,\mathrm{mic}}$ depend linearly on the chain length of alkyl maltosides and cyclohexyl alkyl maltosides. In the absence of urea, the average increments per methylene group are $\partial\Delta G^{\circ,\mathrm{mic}}/\partial n = -3.1$ kJ/mol, $\partial\Delta H^{\circ,\mathrm{mic}}/\partial n = -1.6$ kJ/mol, and $-\partial T\Delta S^{\circ,\mathrm{mic}}/\partial n = -1.6$ kJ/mol for alkyl maltosides as well as $\partial\Delta G^{\circ,\mathrm{mic}}/\partial n = -3.1$ kJ/mol, $\partial\Delta H^{\circ,\mathrm{mic}}/\partial n = -1.8$ kJ/mol, and $-\partial T\Delta S^{\circ,\mathrm{mic}}/\partial n = -1.3$ kJ/mol for cyclohexyl alkyl maltosides. These values agree excellently with $\partial\Delta G^{\circ,\mathrm{mic}}/\partial n = -3.1$ kJ/mol, $\partial\Delta H^{\circ,\mathrm{mic}}/\partial n = -2.0$ kJ/mol, and $-\partial T\Delta S^{\circ,\mathrm{mic}}/\partial \mathrm{n} = -1.0$ kJ/mol reported for various hydrocarbons, alcohols, single-chain amphiphiles, and lysophosphatidylcholines (see ref. (16) and references cited therein).

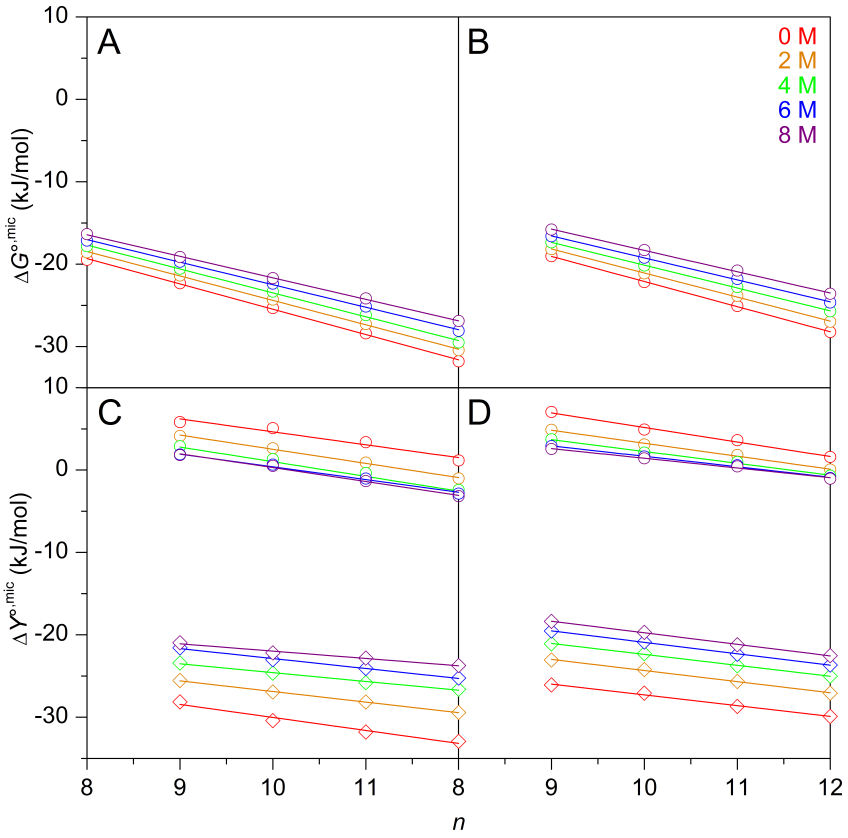

Figure S3. Influence of alkyl chain length, n, on the thermodynamics of micelle formation. (A and C) Alkyl maltosides and (B and D) cyclohexyl alkyl maltosides at different urea concentrations. (Top) Standard molar Gibbs free energy change upon micellization, $\Delta G^{\circ,\mathrm{mic}}$. Experimental data (*open circles*) from Eq. 5 and linear fits (*solid lines*). (Bottom) Standard molar changes (denoted as $\Delta Y^{\circ,\mathrm{mic}}$) in enthalpy, $\Delta H^{\circ,\mathrm{mic}}$ (*open circles*), and entropy, $-T\Delta S^{\circ,\mathrm{mic}}$ (*open diamonds*), obtained from Eqs. 2 and 6, respectively, and linear fits (*solid lines*).

5. Micelle sizes in buffer

To determine the micelle size by DLS, each detergent was used at such a concentration above its CMC that the concentration of micellar detergent was identical at all urea concentrations. For instance, a micellar concentration of 50 mM DDM required a total detergent concentration of 50.15 mM in the absence of urea (where CMC = 0.15 mM) but 51.08 mM at 8 M urea (where CMC = 1.08 mM). The micellar concentration was increased from long- to short-chain detergents to compensate for reduced light scattering. Figure S4 summarizes the results obtained from triplicate DLS measurements on alkyl maltoside (A) and cyclohexyl alkyl maltoside (B) micelles in buffer. The increments in the *Z*-average diameter per methylene group were determined as ~0.45 nm in the case of alkyl maltosides and ~0.60 nm for cyclohexyl alkyl maltosides.

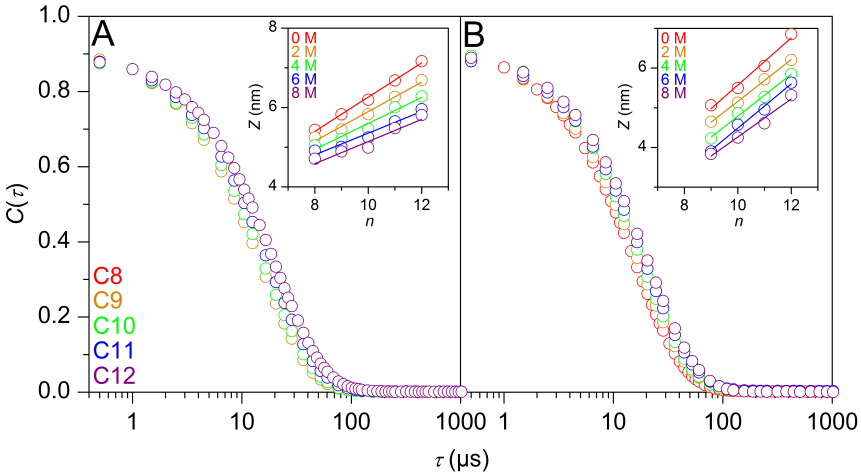

Figure S4. Variation of micelle size with alkyl chain length as assessed by DLS at 25°C. Normalized autocorrelation functions, $C(\tau)$, *versus* delay time, t. (A) Alkyl maltosides and (B) cyclohexyl alkyl maltosides with 8–12 C-atoms in their apolar chains. (Insets) Mean values of the *Z*-average diameter from triplicate measurements as functions of alkyl chain length, n. Experimental data (*open circles*) and linear fits (*solid lines*) for (A) alkyl maltosides and (B) cyclohexyl alkyl maltosides at different urea concentrations.

6. References

(1) Tsamaloukas, A. D., Beck, A., and Heerklotz, H. Modeling the micellization behavior of mixed and pure *n*-alkyl-maltosides. *Langmuir* **2009**, *25*, 4393–4401.

(2) Andersen, K. K., Wang, H., and Otzen, D. E. A Kinetic Analysis of the Folding and Unfolding of OmpA in Urea and Guanidinium Chloride: Single and Parallel Pathways. *Biochemistry* **2012**, *51*, 8371–8383.

(3) He, L., Garamus, V. M., Funari, S. S., Malfois, M., Willumeit, R., and Niemeyer, B. Comparison of small-angle scattering methods for the structural analysis of octyl-*b*-maltopyranoside micelles. *J. Phys. Chem. B* **2002**, *106*, 7596–7604.

(4) Oliver, R. C., Lipfert, J., Fox, D. A., Lo, R. H., Doniach, S., and Columbus, L. Dependence of micelle size and shape on detergent alkyl chain length and head group. *PLoS One* **2013**, *8*, e62488.

(5) Alpes, H., Apell, H. J., Knoll, G., Plattner, H., and Riek, R. Reconstitution of Na^+/K^+-ATPase into phosphatidylcholine vesicles by dialysis of nonionic alkyl maltoside detergents. *Biochim. Biophys. Acta* **1988**, *946*, 379–388.

(6) Walter, A., Kuehl, G., Barnes, K., and VanderWaerdt, G. The vesicle-to-micelle transition of phosphatidylcholine vesicles induced by nonionic detergents: effects of sodium chloride, sucrose and urea. *Biochim. Biophys. Acta* **2000**, *1508*, 20–33.

(7) Lipfert, J., Columbus, L., Chu, V. B., Lesley, S. A., and Doniach, S. Size and shape of detergent micelles determined by small-angle X-ray scattering. *J. Phys. Chem. B* **2007**, *111*, 12427–12438.

(8) VanAken, T., Foxall-VanAken, S., Castleman, S., and Ferguson-Miller, S. Alkyl glycoside detergents: synthesis and applications to the study of membrane proteins. *Methods Enzymol.* **1986**, *125*, 27–35.

(9) Dupuy, C., Auvray, X., Petipas, C., Rico-Lattes, I., and Lattes, A. Anomeric effects on the structure of micelles of alkyl maltosides in water. *Langmuir* **1997**, *13*, 3965–3967.

(10) Lorber, B., Bishop, J. B., and DeLucas, L. J. Purification of octyl-*b*-D-glucopyranoside and re-estimation of its micellar size. *Biochim. Biophys. Acta* **1990**, *1023*, 254–265.

(11) Paula, S., Sues, W., Tuchtenhagen, J., and Blume, A. Thermodynamics of micelle formation as a function of temperature: a high sensitivity titration calorimetry study. *J. Phys. Chem.* **1995**, *99*, 11742–11751.

(12) Antonelli, M. L., Bonicelli, M. G., Ceccaroni, G., Mesa, C., and Sesta, B. Solution properties of octyl-*b* -D-glucoside. Part 2: Thermodynamics of micelle formation. *Colloid Polym. Sci.* **1994**, *272*, 704–711.

(13) Chattopadhyay, A. and London, E. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. *Anal. Biochem.* **1984**, *139*, 408–412.

(14) Krylova, O. O., Jahnke, N., and Keller, S. Membrane solubilisation and reconstitution by octylglucoside: comparison of synthetic lipid and natural lipid extract by isothermal titration calorimetry. *Biophys. Chem.* **2010**, *150*, 105–111.

(15) Zer, H., Vink, M., Shochat, S., Herrmann, R. G., Andersson, B., and Ohad, I. Light affects the accessibility of the thylakoid light harvesting complex II (LHCII) phosphorylation site to the membrane protein kinase(s). *Biochemistry* **2003**, *42*, 728–738.

(16) Heerklotz, H. and Epand, R. M. The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids. *Biophys. J.* **2001**, *80*, 271–279.