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Effect of Detergents on Galactoside Binding by Melibiose Permeases

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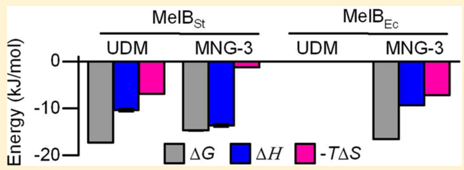
ABSTRACT: The effect of various detergents on the stability and function of the melibiose permeases of *Escherichia coli* ($MelB_{Ec}$) and *Salmonella typhimurium* ($MelB_{St}$) was studied. In *n*-dodecyl-*β*-D-maltoside (DDM) or *n*-undecyl-*β*-D-maltoside (UDM), WT $MelB_{St}$ binds melibiose with an affinity similar to that in the membrane. However, with WT $MelB_{Ec}$ or $MelB_{St}$ mutants (Arg141 → Cys, Arg295 → Cys, or Arg363 → Cys), galactoside binding is not detected in these detergents, but binding to the phosphotransferase protein IIA^{Glc} is maintained. In the amphiphiles lauryl maltose neopentyl glycol (MNG-3) or glyco-diosgenin (GDN), galactoside binding with all of the MelB proteins is observed, with slightly reduced affinities. $MelB_{St}$ is more thermostable than $MelB_{Ec}$, and the thermostability of either MelB is largely increased in MNG-3 or GDN. Therefore, the functional defect with DDM or UDM likely results from the relative instability of the sensitive MelB proteins, and stability, as well as galactoside binding, is retained in MNG-3 or GDN. Furthermore, isothermal titration calorimetry of melibiose binding with $MelB_{St}$ shows that the favorable entropic contribution to the binding free energy is decreased in MNG-3, indicating that the conformational dynamics of MelB is restricted in this detergent.



Membrane transporters, receptors, and channels play crucial roles in cellular functions by moving molecules across cell membranes. Detergents are essential tools for studying the structure and function of membrane proteins; however, selection of a detergent that retains activity and conformational stability is challenging because knowledge about detergents is still limited. The mild, nonionic detergent *n*-dodecyl-*β*-D-maltoside (DDM) is probably the most commonly used detergent for structure determination and functional analysis. The novel amphiphiles lauryl maltose neopentyl glycol (MNG-3) and glyco-diosgenin (GDN) have been shown to be superior to DDM or *n*-undecyl-*β*-D-maltoside (UDM) in maintaining the solubility of several membrane proteins, including the melibiose permease of *Escherichia coli* ($MelB_{Ec}$) and *Salmonella typhimurium* ($MelB_{St}$) as well as G protein-coupled receptors.[1,2] However, their effects on substrate binding affinity and binding thermodynamics to MelB have not been tested yet.

Both $MelB_{Ec}$ and $MelB_{St}$ catalyze symport of galactosides with H^+, Na^+, or Li^+[3−7] and are well-characterized members of the glycoside-pentoside-hexuronide/cation subfamily[8,9] of the major facilitator superfamily of membrane transport proteins.[10−13] The X-ray crystal structure of $MelB_{St}$ shows that MelB is composed of N- and C-terminal domains containing six irregular transmembrane helices surrounding a deep aqueous cavity open to the periplasmic side.[12] This overall fold is similar to that of other major facilitator superfamily transport proteins, such as lactose permease (LacY).[14,15]

$MelB_{St}$ is effectively extracted from membranes with DDM or UDM, and the purified protein in UDM is monodisperse on gel filtration chromatography[12] and does not precipitate when stored at 0 °C for months. $MelB_{St}$ solubilized in UDM binds melibiose,[12,16] nitrophenyl-*α*-galactoside (*α*-NPG),[16] and the fluorescent sugar 2′-(*N*-dansyl)aminoalkyl-1-thio-*β*-D-galactopyranoside (D^2G).[12] Melibiose binding affinity with right-side-out (RSO) membrane vesicles containing WT $MelB_{St}$ or with the purified proteins in UDM is comparable, with a K_d of ~1 mM.[6,12] Surprisingly with UDM-solubilized $MelB_{Ec}$, melibiose reversal in Trp → D^2G fluorescence resonance energy transfer (D^2G FRET) experiments is not detected. Thus far, all Trp → D^2G FRET data with $MelB_{Ec}$ were derived solely from studies with reconstituted proteoliposomes or membrane vesicles.[17−20] In this article, the effect of UDM, DDM, MNG-3, and GDN on ligand binding by $MelB_{Ec}$ or $MelB_{St}$ is characterized by isothermal titration calorimetry and/or D^2G FRET assays. The results indicate that the functional defect with DDM or UDM likely results from relative instability of the sensitive MelB proteins and that MNG-3 or GDN maintains the stability of and galactoside binding with either MelB.

■ MATERIALS AND METHODS

Materials. D^2G was kindly provided by H. Ronald Kaback and Gérard Leblanc. Synthesis of MNG-3 and GDN was described previously.[1,2] DDM, UDM, and DM were purchased from Anatrace. All other materials were reagent grade and obtained from commercial sources.

Plasmids. The expression plasmid pK95ΔAH/WT $MelB_{Ec}$ was from Gérard Leblanc.[21] pK95ΔAH-based plasmids were used for overexpressing WT $MelB_{St}$[6] and $MelB_{St}$ mutants R141C, R295C, or R363C.[13]

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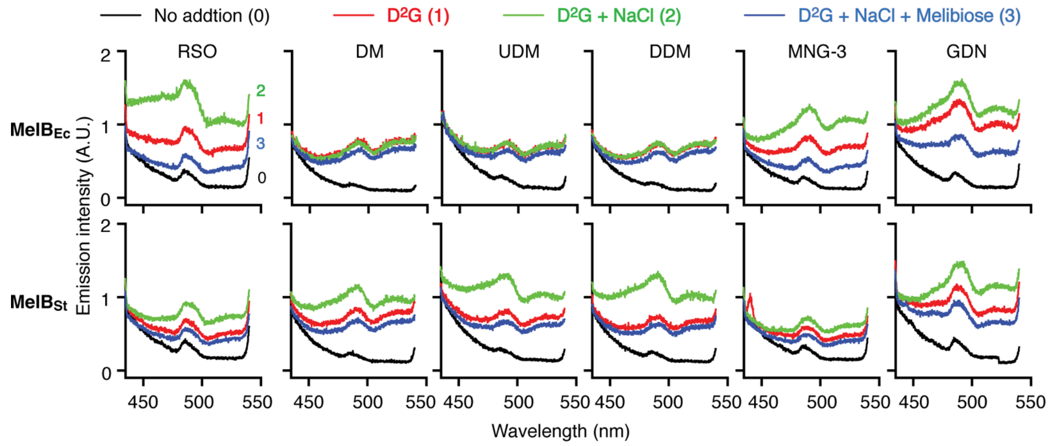

Figure 1. Trp → D^{2}G FRET. RSO vesicles prepared from DW2 cells expressing WT MelB$_{Ec}$ (upper panel) and WT MelB$_{St}$ (lower panel) at a protein concentration of 1.0 mg/mL or solubilized with 1% of the indicated detergent were excited at 290 nm. Emission spectra were recorded between 430 and 550 nm in the absence of (black or curve 0) or presence of 10 μM D^{2}G (red or curve 1), with successive addition of 20 mM NaCl (green or curve 2) and 120 mM melibiose (blue or curve 3). A.U., arbitrary units.

Preparation of RSO Vesicles. RSO membrane vesicles were prepared from *E. coli* DW2 cells by osmotic lysis,[6,22,23] resuspended with 100 mM KP$_i$ (pH 7.5), and stored at −80 °C.

Trp → D^{2}G FRET. RSO membrane vesicles or detergent-solubilized samples were used for Trp → D^{2}G FRET measurements with an Amico-Bowman series 2 (AB2) spectrofluorometer. Trp residues were excited at 290 nm, and emission spectra were recorded between 430 and 550 nm. In time traces, emission was recorded at 465 nm for MelB$_{Ec}$ or 490 nm for MelB$_{St}$. Successive addition of 10 μM D^{2}G, 20 mM NaCl, and excess melibiose was done for all D^{2}G FRET measurements.

Determination of IC$_{50}$ of melibiose for the half-maximal displacement of bound D^{2}G (10 μM) was carried out as described.[6,13,24] Briefly, stepwise addition of melibiose was performed during D^{2}G FRET until no further change in the FRET signal was observed. The IC$_{50}$ was determined by hyperbolic fitting (OriginPro).

MelB Overexpression and Membrane Preparation. Overexpression of MelB and membrane preparation were carried out according to previous protocols.[12]

Thermostability Test. The thermostability of MelB in DDM or GDN was assayed out as described[1,2,25,26] except for the use of 2% detergent concentration. Briefly, membranes containing MelB$_{St}$ or MelB$_{Ec}$ at 10 mg/mL in 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 10% glycerol, and 20 mM melibiose were incubated with 2% (w/v) of DDM or GDN at 0 °C for 10 min and subsequently placed at a given temperature (0, 45, 55, and 65 °C) for 90 min. Samples were ultracentrifuged at 355 590*g* for 45 min at 4 °C. Equal-volume solutions were analyzed by SDS-PAGE and immunoblotted with penta-His-HRP antibody. The thermostability data of WT MelB$_{St}$[27] and MelB$_{Ec}$[1] in MNG-3 have been published.

MelB Purification in Different Detergents. Purification of MelB has been reported.[12] Briefly, *E. coli* DW2 cell membranes at 14 mg/mL were extracted with 1.5% UDM or 1.2% MNG-3, and purified MelB in 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.035% UDM or 0.01% MNG-3, and 10% glycerol was flash-frozen in liquid nitrogen and stored at −80 °C.

Expression and Purification of Phosphotransferase IIA^{Glc}. Expression and purification of IIA^{Glc} of *E. coli* were performed as described.[16,28] Purified IIA^{Glc} in 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 10% glycerol was stored at −80 °C.

Protein Concentration. Protein concentration was determined with the micro BCA protein assay (Pierce Biotechnology, Inc.).

Isothermal Titration Calorimetry. ITC measurements with a nano isothermal titration calorimeter (TA Instruments) and data processing using NanoAnalyze, version 2.3.6, software[29,30] were performed as described.[16,28] MelB in 20 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 10% glycerol, and a given detergent was placed into the sample cell, and melibiose and IIA^{Glc} were prepared in the same buffer used for the permease. Data fitting using a one-site independent binding model[31] yields the association constant (K_a) and enthalpy change ΔH values. The dissociation constant (K_d) = $1/K_a$. Entropy change ($-T\Delta S$) is calculated from the equation $\Delta G = \Delta H - T\Delta S$; $\Delta G = -RT \ln K_a$.

RESULTS

Trp → D^{2}G FRET. D^{2}G is a fluorescent sugar analogue for MelB and LacY.[6,17,18,20,32] As reported,[6,17] with RSO membrane vesicles containing WT MelB$_{Ec}$, Trp → D^{2}G FRET is observed upon addition of D^{2}G (Figure 1, upper left panel, red curve), which is increased by Na$^+$ (green curve) and decreased by consecutive addition of melibiose (blue curve), to a level slightly higher than the background (black curve). The binding affinity for Na$^+$ or melibiose is reflected by Na$^+$ stimulation and melibiose displacement of bound D^{2}G, respectively. With RSO vesicles containing WT MelB$_{St}$, D^{2}G FRET is also obtained,[6] but the FRET signal is less intense (Figure 1, lower left panel). RSO vesicles containing MelB$_{Ec}$ or MelB$_{St}$ bind D^{2}G in the presence of Na$^+$ with a K_d of approximately 3 or 10 μM, respectively, and bind melibiose with a K_d of approximately 0.5 or 1 mM, respectively.[6]

When RSO vesicles containing WT MelB$_{St}$ were solubilized with DDM, UDM, or DM detergent without purification of the protein, a specific D^{2}G FRET signal was also detected (Figure 1). Surprisingly, MelB$_{Ec}$ solubilized with DDM, UDM, or DM does not exhibit Na$^+$ stimulation or melibiose reversal of fluorescent intensity, which is not improved by changing the DDM or UDM concentration from 0.5 to 2.0% (data not

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Table 1. IC_{50} for Melibiose Displacement of Bound D^2G[a] (mM)

permease	RSO membrane vesicles	detergent solubilization DDM	UDM	MNG-3	GDN
$MelB_{Ec}$	0.66 ± 0.16[b]	ND[c]	ND	1.20 ± 0.35	1.82 ± 0.18
$MelB_{St}$	2.42 ± 0.22	5.65 ± 1.75	4.79 ± 0.71	4.22 ± 0.82	3.19 ± 0.41
R295C $MelB_{St}$	4.04 ± 0.42[d]	[e]	[e]	5.49 ± 0.83	[e]

[a]Melibiose concentration for the half-maximal displacement of bound D^2G (IC_{50}) in the presence of 20 mM NaCl. [b]SEM (n = 2). [c]No detectable signal. [d]Data from ref 12. [e]No measurement.

shown). Strikingly, with MNG-3 or GDN, Na^+ stimulation and melibiose reversal of D^2G FRET are obtained with both $MelB_{Ec}$ and $MelB_{St}$ (Figure 1). Notably, the intensity of D^2G FRET is protein- and detergent-dependent in addition to being dependent on the fraction of bound D^2G. The IC_{50} for melibiose displacement of bound D^2G was determined to analyze galactoside-binding affinity. $MelB_{Ec}$ in MNG-3 or GDN and $MelB_{St}$ in each detergent exhibit slightly increased IC_{50} values than those observed with RSO membrane vesicles (Table 1).

Effect of Detergents on $MelB_{St}$ Mutants. Residue R141, R295, or R363 forms multiple interactions between the two domains of $MelB_{St}$[13] for stabilizing its outward conformation.[12] Replacement of R141, R295, or R363 of $MelB_{St}$ with Cys significantly inhibits melibiose uptake with little effect on melibiose or Na^+ binding, as shown by melibiose transport with intact cells and the D^2G FRET assay with RSO vesicles.[13] When R141 or R363 is replaced with Lys, the conservative replacement mutants catalyze melibiose uptake. With RSO vesicles containing mutants R141K or R363K $MelB_{St}$, Na^+ stimulation and melibiose reversal of fluorescent intensity are detected after solubilization in UDM or MNG-3 (Figure 2a). Strikingly, there is no change upon addition of Na^+ or melibiose with RSO vesicles containing $MelB_{St}$ mutants (R141C, R295C, or R363C) after solubilization with UDM (Figure 2b, middle column). When using MNG-3 (Figure 2b, right column), Na^+ stimulation and melibiose reversal of D^2G FRET are observed with all three mutants, although the intensity is weaker than that with RSO membrane vesicles (Figure 2b, left column). With mutant R295C $MelB_{St}$ in MNG-3, the IC_{50} for melibiose displacement of bound D^2G is 5.49 ± 0.83 mM (Table 1).

Sugar Binding by ITC. To determine the K_d value for melibiose binding with MelB purified after solubilization with UDM or MNG-3, ITC measurements were carried out. As shown previously, titration of WT $MelB_{St}$ in UDM with melibiose yields a K_d of 0.97 ± 0.02 mM and ΔG of −17.20 ± 0.07 kJ/mol in the presence of Na^+ (Figure 3, Table 2).[16] Energetically, melibiose binding is driven by both favorable enthalpy (ΔH of −10.33 ± 0.36 kJ/mol) and entropy ($-T\Delta S$ of −6.87 ± 0.28 kJ/mol) (Figure 4b). Remarkably, titration of the WT $MelB_{Ec}$ in UDM with melibiose (10 mM), even at an increased concentration (100 mM), yields a similar heat change to that observed by injection of melibiose into buffer in the absence of protein (Figure 3, right column). Similarly, titration of mutant R141C $MelB_{St}$ with melibiose at 100 mM also exhibits no binding isotherm (Figure 3). These data clearly indicate that WT $MelB_{Ec}$ and mutant R141C $MelB_{St}$ in UDM do not bind melibiose, which correlates well with the D^2G FRET results.

Both WT $MelB_{Ec}$ and $MelB_{St}$ were purified after solubilization with MNG-3. Titration of $MelB_{St}$ in MNG-3 with melibiose exhibits a slightly reduced binding affinity, with a K_d of 2.51 ± 0.13 mM (Figure 4a, Table 2), which is 2.5-fold higher than that in UDM (Table 2). Interestingly, the favorable entropic contribution to the total free energy (ΔG) is largely reduced from 40% in UDM to less than 10% in MNG-3 (Figure 4b, red bars), yielding a $-T\Delta\Delta S_{\text{MNG-3 − UDM}}$ of 5.66 kJ/mol. As a partial compensation, the favorable enthalpic change (ΔH) is increased and contributes to ΔG at greater than 90%. Thus, the decrease in melibiose binding affinity with $MelB_{St}$ in MNG-3 is due solely to a loss of entropy.

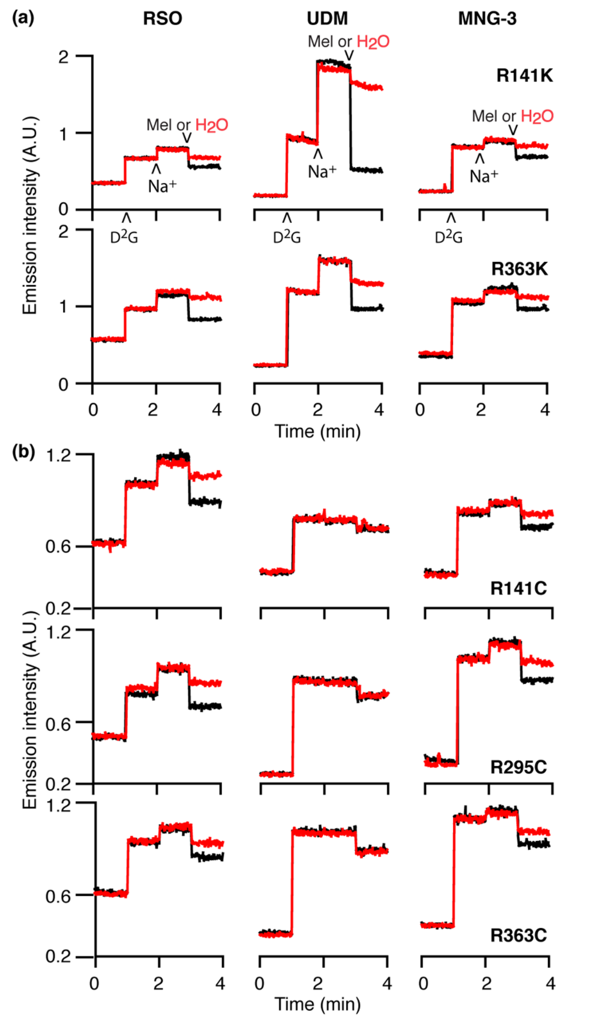

Figure 2. Time trace of Trp → D^2G FRET. With an excitation wavelength of 290 nm, emission was recorded at 465 nm for $MelB_{Ec}$ or 490 nm for $MelB_{St}$. Ten micromolar D^2G, 20 mM NaCl, and melibiose (Mel, black) or water (red) were successively added at 60 s intervals. Left column, recorded with RSO vesicles; middle and right columns, recorded with RSO vesicles after solubilization with 1% UDM or 1% MNG-3, respectively. (a) R141K and R363K $MelB_{St}$ mutants; (b) R141C, R295C, and R363C $MelB_{St}$ mutants.

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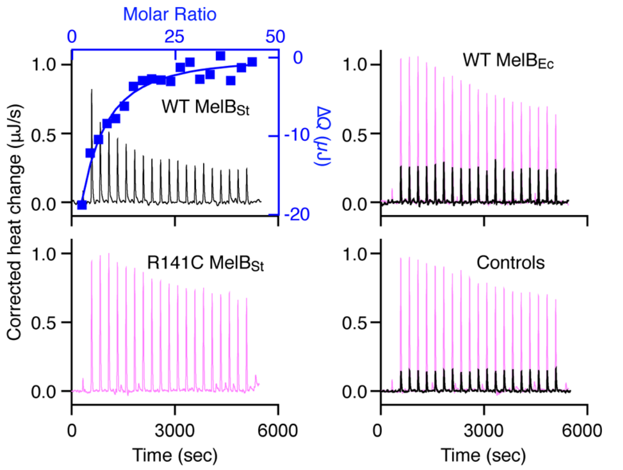

Figure 3. ITC measurement of melibiose binding with MelB in UDM. MelB was extracted with UDM and purified in buffer containing 0.035% UDM. Thermograms (left *y*-axis) were recorded at 25 °C during the titration of WT MelB$_{St}$, mutant R141C MelB$_{St}$, or WT MelB$_{Ec}$ (80 μM) with melibiose at 10 mM (black curves) or 100 mM (pink curves). Injection of melibiose into the buffer in the absence of protein was used for the control. Melibiose binding to WT MelB$_{St}$ has been reported.[12,16] Accumulated heat change (ΔQ, right *y*-axis) of each injection was plotted against the melibiose/MelB molar ratio (on the top) and fitted to a one-site independent binding model.

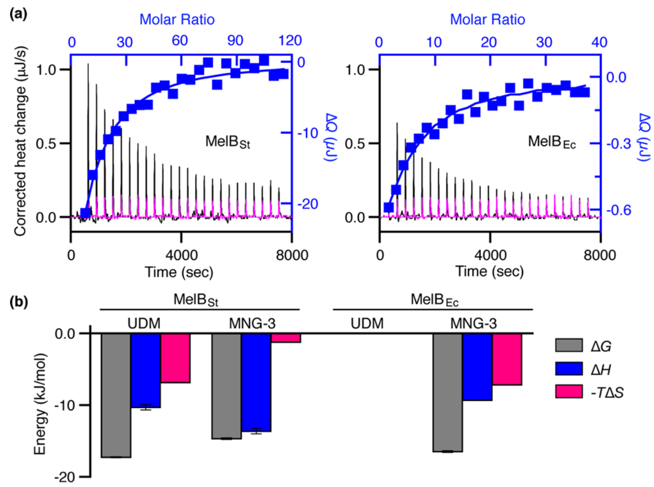

Figure 4. Thermodynamics of melibiose binding with MelB in MNG-3. WT MelB$_{St}$ or MelB$_{Ec}$ was solubilized with MNG-3 and purified in buffers containing 0.01% MNG-3. (a) Thermograms (left *y*-axis) were recorded at 25 °C during the titration of MelB (95 μM) with melibiose at 30 mM (MelB$_{St}$, black curve) or 10 mM (MelB$_{Ec}$, black curve). Injection of melibiose at 30 or 10 mM into the buffer in the absence of protein was used for the control (magenta curves). ΔQ (right *y*-axis) was plotted against the melibiose/MelB molar ratio (on the top) and fitted to a one-site independent binding model. (b) Energetics of melibiose binding with MelB in UDM or MNG-3. Free energy change (ΔG), enthalpy change (ΔH), and entropy change ($-T\Delta S$) are obtained from curve fitting in Figures 3 and in this figure as described in the Materials and Methods. Errors are SEM; number of tests = 2−4.

While no sugar binding is observed with MelB$_{Ec}$ in UDM, titration of MelB$_{Ec}$ in MNG-3 with melibiose reveals a typical binding isotherm with a K_d of 1.28 ± 0.06 mM (Figure 4a), which is about 2.5-fold higher than that measured with RSO by D^{2}G FRET[6] but similar to the K_d obtained with proteoliposomes by flow-dialysis assay using [^{3}H]nitrophenyl-α-D-galactopyranoside.[33] Energetically, both enthalpy and entropy contribute favorably to the free energy, which is similar to melibiose binding with MelB$_{St}$ in UDM (Figure 4b).

IIA^{Glc} Binding by ITC. Previous ITC measurements show that the phosphotransferase IIA^{Glc}, a regulatory protein, binds to MelB$_{St}$,[16] MelB$_{Ec}$,[16] or LacY[28] in UDM, yielding K_d values of ca. 3, 25, or 5 μM, respectively. When melibiose is preincubated with MelB$_{St}$, IIA^{Glc} affinity is 3-fold decreased and the binding rate is faster.[16] However, melibiose has no effect on IIA^{Glc} binding to MelB$_{Ec}$ (Figure 5; also see ref 16), which is now recognized as a lack of melibiose binding. When titrating mutant R141C MelB$_{St}$ in UDM with IIA^{Glc}, a binding curve similar to that of WT MelB$_{St}$ is obtained at a K_d of 2 μM (Figure 5). Again, melibiose shows no effect, which is consistent with a lack of melibiose binding.

Comparison of Thermostability between MelB$_{St}$ and MelB$_{Ec}$. Thermostability studies with MelB$_{St}$ and MelB$_{Ec}$ in DDM, MNG-3, or GDN have been reported,[1,2,27] but the previous focus was not on direct comparison between the two MelB proteins. Data on DDM or GDN (Figure 6) were obtained from similar studies but at higher concentration; the MNG-3 data are from two publications.[1,27] All of the tests were done in the presence of melibiose and NaCl.

DDM quantitatively solubilizes either MelB$_{St}$ or MelB$_{Ec}$ at 0 °C (Figure 6, lanes 1 and 2). After incubation at elevated temperatures (45, 55, or 65 °C) for 90 min, MelB$_{Ec}$ exhibits strong aggregation, as detected by western blot (Figure 6, lanes 3−7). With MelB$_{St}$, only slight aggregation is observed at 45 °C; at 55 °C, the soluble fraction of MelB$_{St}$ is greater than that of MelB$_{Ec}$, as shown with the samples after ultracentrifugation (lane 6); at 65 °C, both MelB proteins disappear from the solutions (lane 8).

MNG-3 or GDN maintains the MelB proteins completely in the soluble fraction after incubation at 55 °C; GDN also keeps all of the MelB protein in solution even after incubation at 65 °C for 90 min (Figure 6).[2] The data indicate that the MelB proteins exhibit increased thermostability in MNG-3 or GDN, by approximately 10 or 20 °C, respectively. In addition, as observed in DDM, MelB$_{St}$ in both MNG-3 and GDN shows less aggregation, clearly indicating that MelB$_{St}$ is more thermostable than MelB$_{Ec}$ (Figure 6).

Table 2. Detergent Effect on Energetics of Melibiose Binding to MelB[a]

permease	K_a (mol^{-1})	K_d (mM)	ΔG (kJ/mol)	ΔH (kJ/mol)	$-T\Delta S$ (kJ/mol)
MelB$_{St}$ in UDM	1030 (27.50)[b]	0.97 (0.02)	−17.20 (0.07)	−10.33 (0.36)	−6.87 (0.28)
MelB$_{St}$ in MNG-3	402.18 (20.60)	2.51(0.13)	−14.68 (0.13)	−13.65 (0.36)	−1.21 (0.40)
MelB$_{Ec}$ in UDM	ND[c]	ND	ND	ND	ND
MelB$_{Ec}$ in MNG-3	782.05 (38.65)	1.28 (0.06)	−16.51 (0.12)	−9.33 (0.04)	−7.18 (0.16)

[a]All measurements were carried out at 25 °C. The data are presented in Figures 3 and 4. [b]SEM; number of tests = 2−4. [c]No detectable signal.

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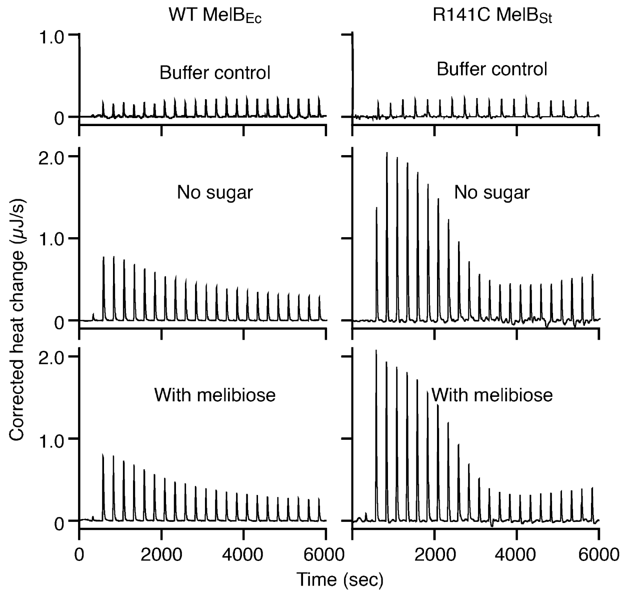

Figure 5. Thermogram of IIA^{Glc} binding. Thermograms following titration of WT $MelB_{Ec}$ (30 μM, 25 °C) or R141C $MelB_{St}$ (50 μM, 20 °C) with IIA^{Glc} (400 or 455 μM) in the absence or presence of 10 mM melibiose were recorded. Both MelB proteins were purified in buffer containing 0.035% UDM after extracted with UDM. Injection of IIA^{Glc} into the buffer in the absence of protein was used as the control. The K_d for IIA^{Glc} binding to WT $MelB_{Ec}$ has been reported.[16]

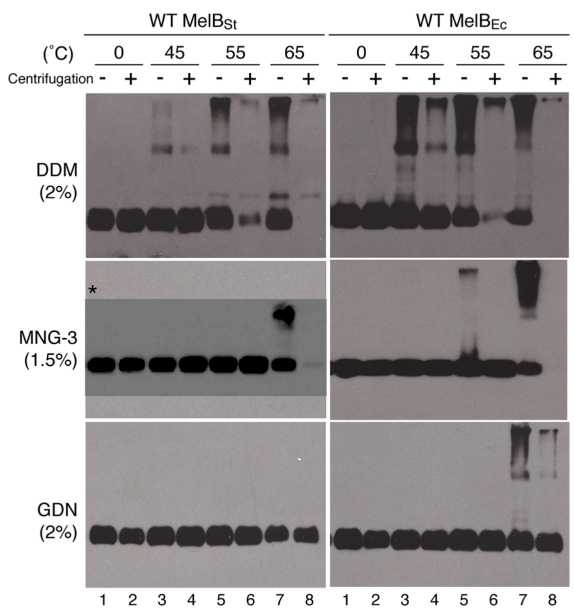

Figure 6. Thermostability. Equal volumes of MelB samples after solubilization with 2% DDM, 2% GDN, or 1.5% MNG-3 in the presence of 20 mM melibiose and 200 mM NaCl without (−) or with (+) ultracentrifugation were loaded onto 12% SDS-PAGE gels. MelB proteins were detected by western blotting using an anti-His tag antibody. Ten micrograms of membrane proteins was used for each test. *, 16% SDS-PAGE gel. The data for MNG-3 have been reported.[1,27]

■ DISCUSSION

$MelB_{St}$ and $MelB_{Ec}$ share more than 85% sequence identity, and the residues necessary for cation and sugar binding are highly conserved.[10] The common detergents DDM and UDM completely extract either permease from the membrane,[1,2] and the purified proteins exhibit no aggregation on ice for weeks to months, but the thermostability of WT $MelB_{St}$ is clearly better than that of WT $MelB_{Ec}$ (Figure 6). Interestingly, DDM and UDM, which work well for $MelB_{St}$, do not support galactoside binding by $MelB_{Ec}$ or by some $MelB_{St}$ mutants (Figures 1−3). Thus, DDM or UDM causes abnormal conformations of these sensitive proteins, but not MeB_{St}; it is likely that these effects are due to the relatively poor stability in these detergents. The effects of DDM and UDM are subtle and reversible without denaturation/aggregation (Figure 6) because the same protein samples bind the regulatory protein IIA^{Glc} (Figure 5). In addition, WT $MelB_{Ec}$, after reconstitution into proteoliposomes, binds Na^+ or Li^+ and galactosides.[17,18,20,34] When using MNG-3 or GDN, galactosides binding with WT $MelB_{Ec}$ and the $MelB_{St}$ mutants is obtained (Figures 1, 2, and 4; Tables 1 and 2). ITC measurement with WT $MelB_{Ec}$ in MNG-3 yields a K_d value of 1.2 mM for melibiose in the presence of NaCl.

The effect of DDM and MNG-3 on a LacY mutant with Cys154 → Gly has also been studied.[35] The C154G mutant LacY, which likely favors an intermediate periplasmic-open conformation in the membrane, collapses to a lower-energy, periplasmic-closed conformation in DDM. Notably, MNG-3 stabilizes C514G LacY in the membrane-embedded form.[35] It has also been reported that the muscarinic acetylcholine receptor requires cholesterol or its derivatives for protein stability and that MNG-3 stabilizes the receptor without cholesterol derivatives.[36] In addition, the off-rate of MNG-3 from the G protein-coupled beta(2)-adrenoreceptor has been shown to be 4 orders of magnitude lower than that of DDM,[37] clearly demonstrating that MNG-3 binds the membrane protein tightly.

Structurally, both MNG-3 and GDN have a branched dimaltoside hydrophilic headgroup,[1,2] which plays an important role in membrane protein stabilization. The two alkyl chains in MNG-3 could have a multivalent effect, binding to membrane proteins tighter than DDM or UDM. GDN has a flat panel-like structure with lipophilic groups and thus likely forms self-assemblies with strong intermolecular interactions around membrane proteins. Both MNG-3 and GDN also have critical micelle concentration (CMC) values (0.001%[1] and 0.002%,[2] respectively) lower than that of DDM (0.008%). While the CMC is only one of many factors determining membrane protein stability, a detergent with a low CMC value has high tendency to form stable micelles, which could contribute to the formation of stable protein−detergent complexes. Thus, the unique structural and biophysical features of these novel amphiphiles and their resulting tight interactions with membrane proteins could be responsible for the increased stability and retention of the galactoside binding of MelB. The tight binding of MNG-3 is supported by thermodynamic characterization using ITC. The results clearly show that the favorable entropy change contributing to melibiose binding with $MelB_{St}$ in MNG-3 is reduced with a $-T\Delta\Delta S_{MNG\text{-}3-UDM}$ of 5.66 kJ/mol. Unfortunately, this parameter for $MelB_{Ec}$ is not available because sugar binding is not observed with $MelB_{Ec}$ in UDM. Loss of entropy implies that the conformational dynamics of $MelB_{St}$ is restricted, likely because MNG-3 interacts tightly with the protein.

Interestingly, melibiose binding with $MelB_{St}$ in MNG-3 exhibits a 2.5-fold increase in the K_d value. The decrease in galactoside binding affinity likely results from the restricted conformational dynamics. Galactoside binding with MelB is

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proposed to use an induced-fit mechanism,[16,28] which is similar to sugar binding in LacY.[38,39] On the basis of this notion, galactoside induces a conformational change in MelB from an open state to an occluded state to form a completely liganded binding site. It has been proposed that IIA^{Glc} inhibits such a process by restricting the conformational entropy of MelB, resulting in largely reduced melibiose binding.[16] It seems that MNG-3 also hinders the induced-fit process in $MelB_{St}$. Thus, for optimal galactoside binding of each MelB, a balance between structural stability and conformational dynamics is needed, which is largely affected by detergent micelles.

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Notes

The authors declare the following competing financial interest(s): P.S.C. is one of co-inventors on a patent that covers MNG-3 and a patent application on GDN.

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■ ABBREVIATIONS

$MelB_{St}$, melibiose permease of *Salmonella typhimurium*; $MelB_{Ec}$, melibiose permease of *Escherichia coli*; *E. coli*, *Escherichia coli*; MNG-3, lauryl maltose neopentyl glycol; GDN, glyco-diosgenin; DDM, *n*-dodecyl-β-D-maltoside; UDM, *n*-undecyl-β-D-maltoside; DM, *n*-decyl-β-D-maltoside; D^2G, 2′-(*N*-dansyl)-aminoalkyl-1-thio-β-D-galactopyranoside; CMC, critical micelle concentration; D^2G FRET, Trp → dansyl-galactoside fluorescence resonance energy transfer; ITC, isothermal titration calorimetry; K_d, dissociation constant; RSO, right-side-out membrane vesicles; ΔQ, accumulated heat change; ΔG, free energy change; ΔH, enthalpy change; $-T\Delta S$, entropy change

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