Further insight into the cosubstrate-induced structural change of the melibiose permease (MelB) of *Escherichia coli* has been sought by investigating the binding and spectroscopic properties of the fluorescent sugar 2′-(N,N-dialkylaminonaphthalene-1-sulfonyl)-aminoethyl 1-thio-β-D-galactopyranoside (Dns-S-Gal) and related analogs (Dns-S-Gal or Dns-S-Gal with a propyl or hexyl instead of an ethyl linker, respectively) interacting with MelB in membrane vesicles or in proteoliposomes. The three analogs efficiently inhibit melibiose transport and bind to MelB in a sodium-dependent fashion. Their dissociation constants (Kd) are in the micromolar range in the presence of NaCl and an order of magnitude higher in its absence. In the presence of NaCl and Dns-S-Gal, sample excitation at 335 or 297 nm gives rise to a fluorescent signal at around 465 nm, whereas Dns-S-Gal or Dns-S-Gal emits a fluorescence light at 490 or 506 nm, respectively. Detailed study of the Dns-S-Gal signal elicited by a 297 nm illumination indicates that a tryptophan-mediated fluorescence resonance energy transfer phenomenon is involved in the response. All fluorescence signals below 500 nm are prevented by addition of melibiose in excess, and the kinetic constants describing their dependence on the probe or NaCl concentrations closely correlate with the probe binding constants. Finally, the Dns-S-Gal signal recorded in sodium-free medium is red shifted by up to 25 nm from that recorded in the presence of NaCl. Taken together, these results suggest (i) that the fluorescence signals below 500 nm arise from Dns-S-Gal molecules bound to MelB, (ii) the presence of a highly hydrophilic environment close to or at the sugar-binding site, the polarity of which increases on moving away from the sugar-binding site, and (iii) that the interaction of sodium ions with MelB enhances the hydrophobicity of this environment. These results are consistent with the induction of a cooperative change of the structure of the sugar-binding site or of its immediate vicinity by the ions.

The melibiose permease (MelB) of *Escherichia coli* is an integral membrane protein that couples the entry of several α-galactosides, such as melibiose or some β-galactosides, to either Na⁺, Li⁺, or H⁺ by a symport or cotransport mechanism (for reviews, see Ref. 1). MelB is encoded by the melB gene and is a member of the super family of homologous sodium/solute symporters (2, 3). It consists of 473 amino acids (molecular mass, 53 kDa), 70% of which are apolar (4, 5). Hydropathy predictions, gene fusion analysis, limited proteolysis, and immunological studies indicate that MelB is a polytopic protein with 12 membrane domains connected by alternated periplasmic or cytoplasmic loops of short or limited size (6–8). A His-tagged melibiose permease has been purified by affinity chromatography and shown to be solely responsible for symport activity in proteoliposomes (5).

Kinetic studies (9), mutagenesis experiments (10–14), and studies of MelB chimeras (15) all suggest that Na⁺, Li⁺, and H⁺ share a common binding site buried, at least in part, in the N-terminal membrane domain of the transporter. This site contains several acidic and polar residues that may be involved in the coordination of either monovalent ions or H₃O⁺ (rather than H⁺) (11). In addition, intrinsic fluorescence studies of a purified His-tagged transporter suggest that cosubstrate binding induces cooperative modifications in MelB conformation (16). Thus, whereas MelB fluorescence emission is slightly quenched by the monovalent ions or moderately enhanced by adding the sugar in a sodium- or lithium-free medium, addition of both substrates induces a large fluorescence change. Subsequent studies have shown that Trp299 and Trp442, located on helices IX and X of MelB, respectively, contribute most of the α-galactoside-induced fluorescence signal increase (17). One or more tryptophans from the N-terminal domain are also involved in the fluorescence response on adding a β-galactoside. Interestingly, the polarity of Trp299 or Trp442 environment decreases upon sugar binding, suggesting that they become less accessible to the solvent. Altogether, these data suggest that the structure of the sugar-binding site, hypothetically located in the C-terminal domain, is directly modified by the sugar and is further modified in a cooperative and indirect fashion upon the binding of the coupling ion to the N-terminal domain.

Further insight into the cosubstrate-induced change of MelB structure can be obtained by probing more directly the sugar-binding site properties with sugar analogs carrying an appropriate reporter. Fluorescent galactosides, such as (N-dansyl-galactopyranoside; Dns-S-Gal, 2′-(N-dansylamino-1-thio-β-D-galactopyranoside; Dns-S-Gal, 2′-(N-dansyl)aminoethyl-1-thio-β-D-galactopyranoside; Dns-S-Gal, 3′-(N-dansylaminopropyl-1-thio-β-D-galactopyranoside; FRET, fluorescence resonance energy transfer; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; em, emission; ex, excitation; RSO, right side out.

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1 The abbreviations used are: MelB, melibiose permease; Mel, melibiose (6-O-α-D-galactopyranosyl-β-D-glucose); aNPG, p-nitrophenyl α-D-glucopyranoside; Dns-S-Gal, 2′-(N-dansylamino-1-thio-β-D-galactopyranoside; Dns-S-Gal, 2′-(N-dansyl)aminoethyl-1-thio-β-D-galactopyranoside; Dns-S-Gal, 3′-(N-dansylaminopropyl-1-thio-β-D-galactopyranoside; FRET, fluorescence resonance energy transfer; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; em, emission; ex, excitation; RSO, right side out.

I. EVIDENCE FOR ION-INDUCED CONFORMATIONAL CHANGE*

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The melibiose permease (MelB) of *Escherichia coli* is an
sylaminooalkyl-1-thio-β-D-galactopyranosides (Dns-S-Gal), seem particularly suited as the dansyl fluorescence properties depend on solvent polarity, providing a means to assess the physicochemical properties of the substrate binding site (or of its immediate proximity) and their variation during catalysis. Previous of the spectroscopic analyses of Dns-S-Gal derivative-Lac carrier interactions in E. coli membranes illustrate this approach (18–21).

In the present study, we show that several Dns-S-Gal derivatives are high affinity ligands of MelB in native membranes or of purified MelB in proteoliposomes. The overlap between protein tryptophan fluorescence emission and Dns-S-Gal absorbance has been exploited to investigate specific aspects of the MelB-cosubstrate interaction using fluorescence resonance energy transfer (FRET) spectroscopy. The results provide information on the environment of Dns-S-Gal molecules bound to MelB. It is shown that the polarity of this environment is modified in a sodium-dependent fashion. Together with cosubstrate-induced intrinsic fluorescence variation data (16, 17), these results are used to speculate on the structural properties of the sugar-binding site of MelB.

**EXPERIMENTAL PROCEDURES**

**Materials**—p-Nitrophenyl-α- or -β-D-galactopyranoside (α-[3H]HNP) and [3H]melibiose were synthesized in our department under the direction of Dr. B. Rousseau. Synthesis of 3-(laurylamido)-N,N′-(dimethylamino)propylene oxide was performed as described by Brandolini et al. (22). Dodecyl maltoside was obtained from Boehringer Mannheim, and the nickel-nitriiloacetic acid (NiNTA) resin was from Qiagen, Inc. The anion exchange resin (Macro-Prep Q support) and Bio-Beads SM-2 were from Bio-Rad. Total E. coli lipids (acetone/ether precipitated) were purchased from Avanti Polar Lipids, Inc. High purity grade chemicals (Suprapur, Merck) were used to prepare media containing less than 20 ppm sodium salts. All other materials were obtained from commercial sources.

The dansyl galactosides Dns-S-Gal, Dns-S-Gal, and Dns-S-Gal were kindly provided by Dr. H. R. Kaback (HHMI/UCLA), and Dns-S-Gal was a gift from Dr. M. Page (Hoffman-LaRoche, Basel, Switzerland). The purity of the different fluorescent compounds was verified by mass spectra and NMR analyses. Stock solutions of the fluorescent derivatives (10 μM) were prepared in dimethyl sulfoxide (DMSO) and stored in frozen vials at −20 °C.

**Bacterial Strains and Plasmids**—A RecA derivative of E. coli DW2 (λmelΔacZ) (23) was transformed with a plasmid expressing wild-type His-tagged-MelB (pK31ΔAHH) (17). Transformed DW2-R cells were grown at 30 °C in M9 medium supplemented with appropriate carbon sources (5) and ampicillin (100 μg/ml).

**Permease Activity**—Right-side-out membrane vesicles were prepared using an osmotic shock procedure (24) and washed in 0.1 M potassium phosphate (pH 7). Transport activity was assessed by measuring the Lac permease activity using the Lac permease gels.

**Fluorescence Measurements**—Steady state fluorescence measurements were performed with a LS50B Perkin-Elmer spectrophotometer equipped with 1-cm-path length cuvettes, subjected to magnetic stirring, and thermostatted at 20 °C. Experiments were carried out on briefly sonicated proteoliposomes resuspended at 15 μg of protein/ml in Na+-free, 100 mM potassium phosphate buffer (pH 7) and preequilibrated at 20 °C. Each emission fluorescence spectrum is the mean of three scans recorded at 0.5-nm step intervals and with an integration rate of 1 nsec. All illustrated spectra are corrected for the baseline signal recorded in the absence of Dns-S-Gal. When experiments were carried out on sonicated liposomes, the liposome concentration was adjusted to give a final absorbance similar to that of the proteoliposome suspension (A400 about 0.1). The specific spectral contribution of Dns-S-Gal interacting with MelB (λem,max) at 465 nm was corrected for nonspecific contribution using the signal recorded in a NaCl-free medium containing melibiose in excess (100 μM).

**FRET Studies of Mel Permease**

**RESULTS**

**Dansylated Galactosides Are Substrates of Melibiose Permease**—Dansylaminooalkyl-1-thio-β-D-galactopyranosides, with an alkyl linker between the galactoside and dansyl moiety containing 2, 3, and 6 carbons, respectively (reviewed in Ref. 20). Fig. 1A shows that the sodium-dependent accumulation of [3H]melibiose in energized RSO-membrane vesicles from E. coli DW2/RpK31ΔAHH is inhibited by 70 and 50% in the presence of Dns-S-Gal and Dns-S-Gal, respectively, at a final concentration of 10 μM, and by only 30% in the presence of 20 μM Dns-S-S-Gal. Inhibition of MelB activity by the different Dns-S-Gal derivatives was further documented by looking at their effects on the sodium-dependent binding of a high affinity sugar analogue (α-[3H]HNP) to deenergized membrane vesicles. α-[3H]HNP binding was measured in the absence or presence of Dns-S-S-Gal at a final concentration of 5 μM, and a Scatchard plot was made of the data (Fig. 1B). The results indicate that Dns-S-S-Gal behaves as
The fluorescence signal recorded from MelB proteoliposomes was analyzed in the 310–570 nm interval to assess the implication of permease Trps in the Dns-2-S-Gal fluorescence signal upon illumination at 297 ± 5 nm (Fig. 4A). In the absence of Dns-2-S-Gal and NaCl, the fluorescence contribution in the 310–400 nm interval is typical of the transporter Trps (Fig. 4A, spectrum a) (16). Addition of 15 μM Dns-2-S-Gal gave rise to a small signal between 420 and 540 nm and a peak at 540 nm typical of dansylated sugars in an aqueous medium (Fig. 4A, spectrum b). The Trps fluorescence signal also decreased, in part because of an inner filter effect and a nonspecific quenching of the Trp signal by Dns-2-S-Gal from the medium (data not shown). Subsequent addition of 10 mM NaCl

![Fig. 2. Emission fluorescence properties of Dns-2-S-Gal incubated with DW2/pK95ΔAHB RSO membrane vesicles.](http://www.jbc.org/content/394/23/13788/fig/2)

![Fig. 3. Comparative emission fluorescence properties of Dns-2-S-Gal, Dns-3-S-Gal, and Dns-6-S-Gal incubated in the presence of DW2/pK95ΔAHB RSO membrane vesicles.](http://www.jbc.org/content/394/23/13788/fig/3)
induced not only a drastic increase in light emitted between 420 and 540 nm but at the same time a quenching of the Trps fluorescence signal (Fig. 4A, spectrum c). Finally, these spectral changes were reversed by adding melibiose in excess (100 mM) (Fig. 4A, spectrum d), i.e. the 420–540 nm signal decreases, and at the same time, the Trps signal increased or even exceeded the level recorded before addition of NaCl. The calculated difference spectrum (spectra c–d) (i.e. ± melibiose) has a maximum at 465 nm (Fig. 4A, inset). Fig. 4B shows that no such fluorescence signal changes were observed in liposome suspensions. Finally, it is worth mentioning that the sodium-depend-ent increase in fluorescence signal recorded at 465 nm (± 5 nm) has an excitation spectrum with two distinct maxima (not shown). That at 335 nm is proportionally small, NaCl-insensitive, and overlaps the Dns 2-S-Gal absorption spectrum. The other maximum (285 nm) is about 3 times greater, overlaps the spectra recorded before and after addition of melibiose. B, sonicated E. coli phospholipids (used to prepare the proteoliposomes) were incubated in Na + -free 10 mM potassium phosphate (pH 7) and the appropriate ionophores (see Fig. 1B) at 20 °C. The sample was illuminated at 295 nm (± 5 nm), and the emission fluorescence was recorded between 310 and 570 nm before any addition (a) or after the consecutive additions of Dns 2-S-Gal at 15 μM (b), 10 mM NaCl (c, heavy line), and finally 100 mM melibiose added as dry powder (d, dotted line). Each spectrum is the mean of three scans. Inset, specific MelB bound Dns 2-S-Gal FRET signal calculated from the difference between the spectra recorded before and after addition of melibiose. B, sonicated E. coli phospholipids (used to prepare the proteoliposomes) were incubated in Na + -free 10 mM potassium phosphate (pH 7) and the appropriate ionophores. Their concentration was adjusted to give an absorbance at A 280 similar to that of the proteoliposome suspension used above. Spectra a and b were recorded before and after addition of Dns 2-S-Gal at 15 μM, respectively.

FIG. 4. Evidence of a FRET phenomenon between MelB tryptophans and Dns 2-S-Gal in proteoliposomes. A, sonicated proteoliposomes harboring purified His 6-tagged MelB (15 μg) were equilibrated in 1 ml of nominally Na + -free 10 mM potassium phosphate (pH 7) and the appropriate ionophores (see Fig. 1B) at 20 °C. The sample was illuminated at 295 nm (± 5 nm), and the emission fluorescence was recorded between 310 and 570 nm before any addition (a) or after the consecutive additions of Dns 2-S-Gal at 15 μM (b), 10 mM NaCl (c, heavy line), and finally 100 mM melibiose added as dry powder (d, dotted line). Each spectrum is the mean of three scans. Inset, specific MelB bound Dns 2-S-Gal FRET signal calculated from the difference between the spectra recorded before and after addition of melibiose. B, sonicated E. coli phospholipids (used to prepare the proteoliposomes) were incubated in Na + -free 10 mM potassium phosphate (pH 7) and the appropriate ionophores. Their concentration was adjusted to give an absorbance at A 280 similar to that of the proteoliposome suspension used above. Spectra a and b were recorded before and after addition of Dns 2-S-Gal at 15 μM, respectively.

Characterization of the (Trp → Dns)-FRET Phenomenon—In MelB proteoliposomes incubated in the presence of a concentration of NaCl that fully activates MelB (10 mM), the specific (Trp → Dns)-FRET signal saturated as a function of Dns 2-S-Gal (Fig. 5). Half maximal signal variation was reached at a Dns 2-S-Gal concentration of 1.5 μM (Fig. 5B). This value compares well with the Dns 2-S-Gal K d value determined by binding studies (Table I). In addition, the concentration of NaCl that produces half maximal activation of the (Trp → Dns)-FRET signal (K 0.5 [act] = 0.05 mM) is in satisfactory agreement with that producing half maximal activation of the sugar-induced intrinsic fluorescence change (K 0.5 [act] = 0.1 mM) or with the Na + -activation constant for [ 3 H]NPG binding (0.2 mM) (16). Finally, the signal recorded in the presence of 1 or 5 mM Dns 2-S-Gal and 10 mM NaCl was measured in the presence of increasing concentrations of melibiose (range, 0.5–40 mM). Kinetic analysis of the data suggests that Dns 2-S-Gal and melibiose binding processes are mutually exclusive (not shown). The calculated melibiose inhibitory constant (K i = 1.3 mM) is of the same order of magnitude as that determined in binding assays carried out in the presence of the specific high affinity ligand α-[ 3 H]NPG (9).

Acylation of MelB cysteine(s) by N-ethylmaleimide impairs the translocation but not the binding of MelB substrates (10). Exposing membrane vesicles or proteoliposomes to 0.5 mM of NEM for 30 min had no significant effect on the characteristics of the sodium-dependent (Trp → Dns)-FRET signal (not shown). These data indicate that the (Trp → Dns)-FRET phenomenon is associated with events occurring at the stage of substrate binding to the permease.

Sodium-induced Change in the Microenvironment of Dns 2-S-Gal Bound to MelB—The results shown in Fig. 4 indicate that the (Trp → Dns)-FRET signal from MelB proteoliposomes is much more important in the presence of 10 mM NaCl than in its absence. To assess whether the sodium-induced signal variation is cumulative to a modification of the energy donor (MelB Trps) and/or acceptor (bound Dns 2-S-Gal) properties, we analyzed the fluorescence signal elicited by an excitation at 335 or at 297 nm in media before and after addition of NaCl (Fig. 6). For this comparison, the fraction of permeases interacting with Dns 2-S-Gal was kept constant by incubating the proteoliposomes in the presence of 1.5 μM Dns 2-S-Gal in NaCl-containing medium or 15 μM in sodium-free medium, i.e. at analog concentration corresponding to the binding constant in each medium. At both excitation wavelengths (Fig. 6, A and B), the fluorescence signal amplitude was much higher in the sodium-containing (upper spectra) than in the sodium-free (lower spectra) solution. More important, the λ em (max) signal in sodium-free medium was about 490 or 475 nm upon direct or indirect excitation, respectively (Fig. 6, A and B, lower traces), whereas

FIG. 5. Dependence of the FRET signal in MelB proteoliposomes on the concentration of Dns 2-S-Gal. Fluorescence resonance energy transfer signals were measured as described in Fig. 4 (A, 297 (± 5 nm), λ em, 310–570 nm) using proteoliposomes (15 μg) equilibrated in 1 ml of 0.1 mM potassium phosphate (pH 7) containing 20 mM NaCl, the appropriate ionophores, and a concentration of Dns 2-S-Gal varying within the range of 0.5–10 μM. A, specific contribution of MelB bound Dns 2-S-Gal to the FRET signal. The specific FRET signal at any given Dns 2-S-Gal concentration was calculated by subtracting the fluorescence spectrum measured in a Na + -free medium containing 100 mM melibiose from that measured in the presence of 20 mM NaCl. B, Lineweaver-Burk representation of the integrated specific fluorescence contribution (F) of Dns 2-S-Gal as a function of the dansylated sugar concentration (data taken from A).
DISCUSSION

The experiments reported in the present study show that additional insight into the cosubstrate-induced structural change of the MelB transporter of *E. coli* can be obtained by analyzing the spectral properties of different fluorescent 2'-[N-dansyl] aminooalkyl 1-thio-β-D-galactopyranosides interacting with MelB in membrane vesicles or proteoliposomes. The results suggest that on interacting with MelB, the activating sodium ion modifies the hydrophobicity of the immediate environment of the transporter sugar-binding site, probably as a result of structural change occurring in domains close to or forming this sugar-binding site.

The dansylated sugars Dns²-S-Gal, Dns³-S-Gal, and Dns⁶-S-Gal fulfill all of the criteria required for being considered high affinity substrates of MelB. At the micromolar level, they induced active transport of melibiose (Fig. 1A) or competed with the high affinity ligand α-NPG for binding on MelB in deenrized RSO membrane vesicles (Fig. 1B). In addition, they bound to MelB in a sodium-dependent fashion (Table I) and were displaced by melibiose in a competitive-like fashion. The affinity of MelB for the Dns-S-Gal analogs is 2 orders of magnitude higher than that of the physiological substrate melibiose (around 0.5 mM) but is comparable to that of α-NPG (0.6 μM). In agreement with previous contention, the data suggest that although the specificity of the sugar-MelB interaction is primarily dictated by the galactosyl configuration, grafting an aromatic group on the glycosidic linkage strongly stabilizes the sugar-transporter interaction (see Ref. 1 and references there-in). Overall, these data strongly support the contention that Dns-S-Gal analogs bind specifically to the sugar-binding site of MelB.

Two lines of evidence indicate that Dns-S-Gal molecules specifically bound to the transporter are responsible for the generation of the fluorescence signal with λ<sub>em</sub> (max) below 500 nm recorded on irradiating membrane vesicles or MelB proteoliposomes at either 335 or 297 nm. The first is kinetic and is illustrated by the close correlation existing between the spectral and binding properties of Dns²-S-Gal. Both processes are sodium-dependent, progressively titrated by increasing concentration of melibiose, and described quantitatively by using similar kinetic constants. As selective inactivation of the substrate or on the fluorescence response means that the fluorescence signal monitors events occurring at the stage of substrate binding to the permease. The spectral characteristics of the sodium-dependent fluorescence signal also suggest a direct relationship between the spectroscopic and Dns-S-Gal binding to MelB. Thus, the λ<sub>em</sub> (max) value recorded in the presence of Dns²-S-Gal (465 nm) or even that of Dns³-S-Gal (490 nm), is far below that attributed to the same analog interacting nonspecifically with *E. coli* membranes or lipids (514–520 nm) (21). Also, one would not expect much change in the emission maximum of different sugar analogs nor a strong dependence on the

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**Fig. 6. Effect of NaCl on Dns²-S-Gal spectral properties.** Sonicated proteoliposomes harboring purified His<sub>e</sub>-tagged MelB (15 μg) were equilibrated in 1 ml of nominally Na<sup>+</sup>-free medium containing 10 mM potassium phosphate (pH 7) and ionophores as described in the legend of Fig. 1B. The following additions were made: 15 mM Dns²-S-Gal and no NaCl added, dotted traces; 1.5 mM Dns²-S-Gal and 10 mM NaCl, continuous traces. Samples were illuminated at either 335 ± 5 nm (A) or 295 ± 5 nm (B), and the emission fluorescence was recorded between 400 and 570 nm. In each condition, the specific fluorescence emission signal from bound Dns²-S-Gal was calculated from the difference between the spectra recorded before and after addition of melibiose at a final concentration of 100 mM. Maximal fluorescence emission values are indicated by arrows.

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**Fig. 7. Deconvolution of the specific fluorescence emission signals from bound Dns²-S-Gal recorded in Na<sup>+</sup>-free medium on illuminating the samples at 335 nm (A) or 297 nm (B).** Spectra a, recorded spectra taken from Fig. 6; spectra b, Na<sup>+</sup>-dependent component of the spectral responses due to the presence of contaminating sodium salts at a concentration of 15 mM. Its amplitude was calculated using 0.05 mM as a concentration of NaCl producing half maximal activation (K<sub>a</sub>[act]) of the fluorescence signal. Spectra c, H<sup>+</sup>-dependent component of the spectral responses calculated by correcting each recorded spectrum for the contribution of the Na<sup>+</sup>-dependent component.
presence of NaCl if the fluorescent signal were to arise from dansyl galactosides partitioned into the membrane lipid core or not specifically interacting with MelB. Incidentally, it should be noted that the $\lambda_{em}^{\text{max}}$ of the fluorescence signal emitted by Dns$^2$-S-Gal bound to E. coli Lac permease is around 493 nm rather than 465 nm as for MelB (18, 21), indicating that the signal is specific to the transporter studied. Finally, the com- consitituent and reciprocal variation of Trp and Dns$^2$-S-Gal fluorescence signals in proteoliposomes illuminated at 297 nm and the appearance of the 280-nm component in the dansylated sugar excitation spectrum support the conclusion that the fluores- cence signal recorded at 465 nm results from a fluorescence resonance energy transfer phenomenon (Trp $\rightarrow$ Dns-FRET) between the tryptophan residues of MelB and the probe. Emission of Dns$^2$-S-Gal at about 465 nm in the presence of NaCl suggests that the dansyl reporter is located in a highly hydro- phobic microenvironment with an apparent dielectric constant equivalent to that of cyclohexane ($\varepsilon \approx 2.1, (28)$). As the alkyl linker bridging the galactoside and the dansyl reporter of Dns$^2$- S-Gal is short (2 carbons), the microenvironment probed should be in the immediate vicinity of the galactosyl binding site. On using analogs with increasing alkyl bridge lengths (Dns$^n$-S-Gal or Dns$^4$-S-Gal), the signal $\lambda_{em}^{\text{max}}$ was progressively shifted toward higher wavelengths, suggesting that the dansyl is pro-gressively displaced toward a less hydrophobic environment. In this context, Zani et al. (14) proposed a structural model of MelB consisting of a well-like structure open toward the periplasmic space and closed at the bottom by the cosubstrate binding and/or translocation domain in close contact with the cytoplasm. The data reported here might indicate that the pathway becomes less polar near the bottom of the well. Such a polarity gradient could vanish over a restricted distance and could partially shield the sugar ligands in the site from the solvent. Interestingly, a similar situation seems to prevail at the metal binding site of metalloproteins (29) or at the $H^+$ binding site in the membrane sector of $H^+$-transporting ATPases (30). Yamashita et al. (29) pointed out that the metal ion binding sites of most metalloproteins are surrounded by an hydrophobic environment and introduced the concept of hydro- phobic contrast. They derived an hydrophobic contrast function that can be used to predict the location of the metal binding site in metalloproteins structures. Importantly, they considered that the function describing the hydrophobic contrast in metal ion sites “may indirectly represent electrostatic and hydration components of the free energy of metal binding” (29). Extrapolating this notion to the binding of any hydrophilic substrate, we speculate that the hydrophobic contrast between the MelB sugar-binding site and its surroundings that is illustrated in this study has an analogous functional implication.

The Dns$^2$-S-Gal signal amplitude is small and has a $\lambda_{em}^{\text{max}}$ of about 490 nm in a nominally sodium-free medium, whereas the signal is much larger and has a $\lambda_{em}^{\text{max}}$ of 465 nm in NaCl-containing media. The shift of $\lambda_{em}^{\text{max}}$ toward a lower wavelength observed on adding NaCl suggests that the acti-vating monovalent cation enhances the hydrophobicity of the microenvironment of Dns$^2$-S-Gal bound to MelB. The resulting increase of the probe quantum yield accounts for at least part of the signal amplitude enhancement. The NaCl-induced change in Dns$^2$-S-Gal microenvironment polarity most likely reflects a variation of MelB structure close to or at the sugar-binding site. This suggestion is consistent with previous evidence indicating that the structure of MelB is modified in the presence of Na$^+$ ions (8),(16, 17). Another interesting finding is that the ratio of the (Trp $\rightarrow$ Dns)-FRET and direct fluorescence signal is twice as large in the presence as in the absence of Na$^+$ ion, suggesting that more light is absorbed by the acceptor when Na$^+$ ions rather than H$^+$ are bound to the transporter. This effect may be due to a change in the orientation of donor Trp(s) and/or of the acceptor Dns$^2$-S-Gal caused by modification of their respective local environment and/or structure. Ion-induced modification of MelB structure could also change the donor(s)-acceptor distance. Better understanding of these spectroscopic events and possible structural implications requires the prior identification of the tryptophanyl residues acting as energy donors in the (Trp $\rightarrow$ Dns)-FRET phenomenon. In the accompanying paper (32), we used a site-directed mutagenesis approach to assess the participation of each of the eight Trps of MelB to the (Trp $\rightarrow$ Dns)-FRET signal.

REFERENCES

1. Pourcher, T., Bassilana, M., Sarkar, H. K., Kaback, H. R., and Leblanc, G. (1990) Philos. Trans. R. Soc. Lond. B Biol. Sci. 326, 411–423
2. Reizer, J., Reizer, A., and Saier, M. H., Jr. (1994) Biochim. Biophys. Acta 1233, 133–166
3. Poolman, B., and Konings, W. N. (1993) Biochim. Biophys. Acta 1183, 5–39
4. Yazyu, H., Shiota-Niya, S., Shimamoto, T., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. 259, 4320–4326
5. Pourcher, T., Leclercq, S., Brandolin, G., and Leblanc, G. (1995) Biochemistry 34, 4412–4420
6. Botfield, M. C., Naguchi, K., Tsuchiya, T., and Wilson, T. H. (1992) J. Biol. Chem. 267, 1818–1822
7. Pourcher, T., Bibi, E., Kaback, H. R., and Leblanc, G. (1996) Biochemistry 35, 4161–4168
8. Gwizdek, C., Leblanc, G., and Bassilana, M. (1997) Biochemistry 36, 8522–8529
9. Damiano-Ferano, E., Bassilana, M., and Leblanc, G. (1986) J. Biol. Chem. 261, 6893–6899
10. Pourcher, T., Deckert, M., Bassilana, M., and Leblanc, G. (1991) Biochim. Biophys. Res. Commun. 178, 1176–1181
11. Pourcher, T., Zani, M. L., and Leblanc, G. (1993) J. Biol. Chem. 268, 3209–3215
12. Wilson, D. M., and Wilson, T. H. (1992) J. Bacteriol. 174, 3083–3086
13. Zani, M. L., Pourcher, T., and Leblanc, G. (1993) J. Biol. Chem. 268, 3216–3221
14. Zani, M. L., Pourcher, T., and Leblanc, G. (1994) J. Biol. Chem. 269, 24883–24889
15. Hama, H., and Wilson, T. H. (1994) J. Biol. Chem. 269, 1063–1067
16. Mus-Veteau, I., Pourcher, T., and Leblanc, G. (1995) Biochemistry 34, 6775–6783
17. Mus-Veteau, I., and Leblanc, G. (1996) Biochemistry 35, 12053–12060
18. Schuldiner, S., Kerwar, G. K., Kaback, H. R., and Weil, R. (1975) J. Biol. Chem. 250, 1361–1370
19. Schuldiner, S., Kung, H., Kaback, H. R., and Weil, R. (1975) J. Biol. Chem. 250, 3679–3682
20. Schuldiner, S., and Kaback, H. R. (1977) Biochim. Biophys. Acta 472, 399–418
21. Overath, P., Teather, R. M., Simonet, R. D., Aichele, G., and Wilhelm, U. (1979) Biochemistry 18, 1–11
22. Brandolin, G., Doussiere, J., Galik, A., Galik-Krzywicki, T., Lauquin, G. J., and Vignais, P. V. (1980) Biochim. Biophys. Acta 592, 592–614
23. Botfield, M. C., and Wilson, T. H. (1988) J. Biol. Chem. 263, 12909–12915
24. Kaback, H. R. (1971) Methods Enzymol. 23, 99–120
25. Bassilana, M., Pourcher, T., and Leblanc, G. (1987) J. Biol. Chem. 262, 18655–18670
26. Rigaud, J. L., Paternostre, M. T., and Bluzat, A. (1988) Biochemistry 27, 2677–2684
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
28. Chen, R. F. (1967) Arch. Biochem. Biophys. 120, 609–620
29. Yamashita, M. M., Wesson, L., Eisenman, G., and Eisenberg, D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5648–5652
30. Fillingame, R. H. (1996) Curr. Opin. Struct. Biol. 6, 491–498
31. Rosset, B., Vuilleumier, P., Gachet, C., Balerna, M., and Lazdunski, M. (1980) J. Biol. Chem. 255, 9936–9941
32. Cordat, E., Mus-Veteau, I., and Leblanc, G. (1998) J. Biol. Chem. 273, 33193–33202

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