A symporter's secrets shown

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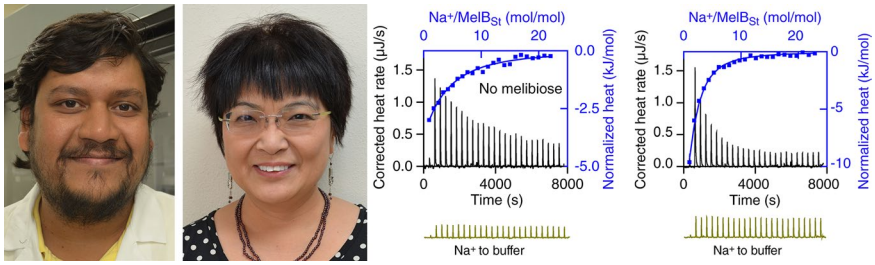
New JGP study explores the thermodynamic cycle and cation preference of the sugar symporter MelB.



Authors Parameswaran Hariharan (left) and Lan Guan (right) provide the first direct examination of Na^+ binding to MelB in the absence (left) and presence (right) of melibiose, allowing them to describe the protein's entire thermodynamic cycle. PHOTOS COURTESY OF THE AUTHORS.

Like our own cells, bacteria devote enormous effort to seeking out food, particularly carbohydrates, which are used in a variety of metabolic pathways. Before nutrients can be used, however, they must first be transported across the cell membrane. That's where sugar transporters such as the bacterial melibiose transporter (MelB) come in. A paper by Parameswaran Hariharan and Lan Guan, published this month in JGP, offers important new details about how this transporter works (1).

MelB is a member of the major facilitator superfamily (MFS) of transporters. Most bacterial MFS proteins couple transport of a sugar or other compound with that of ions, usually protons (H^+), because H^+ tends to flow down its electrochemical gradient into the cell. Transport can be coupled in either the same (symport) or the opposite (antiport) direction as ion flow. A symporter, MelB can use H^+ to drive uptake of the galactoside melibiose. However, in some bacteria including *Escherichia coli* and *Salmonella typhimurium*, it primarily uses sodium (Na^+) instead of H^+ and can even use lithium (Li^+; 2). All three of these cations bind to the same site on MelB, located near the melibiose-binding site (3).

"From a biophysical point of view, it's very interesting that this single site is available for all three cations," notes Lan Guan, an Associate Professor at Texas Tech University Health Sciences Center. "How does the protein select the cation, and which is better for transport?"

Prior studies have demonstrated that binding of melibiose to *E. coli* MelB ($MelB_{Ec}$) is improved in the presence of Na^+ (4), but researchers couldn't directly study cation binding because purified $MelB_{Ec}$ is unstable without its ligands. However, Guan and colleagues recently discovered that *S. typhimurium* MelB ($MelB_{St}$) is more stable and even succeeded in obtaining a crystallographic structure of it (5).

"Then, one day I got a surprise from my postdoc Parameswaran, the first author on this study. He told me he could obtain stable $MelB_{St}$ protein in the absence of Na^+ and melibiose," says Guan.

> "How does the protein select the cation, and which is better for transport?"

Hariharan's breakthrough allowed the researchers to directly measure binding of Na^+ and melibiose, independently or together, using a sensitive technique called isothermal titration calorimetry. Their data showed that $MelB_{St}$ binds Na^+ alone fairly well but has less affinity for melibiose alone. Importantly, though, Na^+ binding improves melibiose binding about eightfold, while melibiose also enhances Na^+ binding eightfold, suggesting that binding of the two ligands to $MelB_{St}$ is cooperative. Furthermore, regardless of whether the cation or the sugar binds to MelB first, the reaction liberates ~35 kJ/mol of energy, which could be used to fuel the conformational transformation that transports the cargoes across the membrane.

Next, the authors explored the relative affinity of $MelB_{St}$'s cation-binding site for Na^+ and H^+. To their surprise, they found that $MelB_{St}$ bound H^+ with 1,000-fold greater affinity than Na^+. It is only by virtue of Na^+'s greater environmental abundance that Na^+ normally outcompetes H^+ for binding to $MelB_{St}$—and if Na^+ is not available, $MelB_{St}$ can fall back on H^+ to drive melibiose transport. This same selection mechanism is used in another membrane protein with similar cation specificity (6).

Hariharan and Guan also examined how Na^+ binds to $MelB_{St}$. They determined that binding of Na^+ displaces a proton from MelB and that only ~20% of $MelB_{St}$ is protonated at pH 7.45. Without Na^+ or Li^+, only protonated $MelB_{St}$ can perform the coupled transport, and the cooperativity of melibiose binding is lower for H^+ than for Na^+. This may explain why earlier studies found that melibiose binding and transport when using H^+ are relatively poor.

These new insights into the $MelB_{St}$ thermodynamic cycle and its cation selectivity should help researchers gain better understanding of other transporter proteins. In fact, Guan is already studying MelB from other bacteria and hopes to have more data to share soon.

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