Locating an extracellular K\(^+\)-dependent interaction site that modulates betaine-binding of the Na\(^+\)-coupled betaine symporter BetP

Lin Ge\(^{a,1}\), Camilo Perez\(^{b,1}\), Izabela Waclawska\(^a\), Christine Ziegler\(^{a,2}\), and Daniel J. Muller\(^{a,2}\)

\(^a\)Department of Biosystems Science and Engineering, Eidgenössische Technische Hochschule (ETH) Zurich, 4058 Basel, Switzerland; and \(^b\)Department of Structural Biology, Max-Planck-Institute of Biophysics, 60528 Frankfurt am Main, Germany

AUTHOR SUMMARY

BetP is a transport protein found in the inner cellular membrane of the bacterium Corynebacterium glutamicum. Specifically, BetP transports one zwitterionic betaine—an important compound that promotes protein folding and protects the cell against osmotic and cold stresses—together with two sodium ions in the same direction across the cell membrane. BetP regulates the rate of betaine transport according to the concentration of the surrounding solution via a sophisticated network of interactions within and between the individual protomers, of the BetP trimer (1). Based on a wealth of functional and structural data, BetP is a paradigm for regulated transporters (2). Under conditions in which the solute concentration is normal (isotonic) or low (hypotonic), BetP shows nearly no activity. However, the protein responds to high osmolality, which results in hyperosmotic stress and instant regulation of its transport activity. In brief, two types of stimuli trigger the regulation of BetP activity: \(i\) an increased K\(^+\) concentration above a threshold of 100 mM in the bacterial cytoplasm and \(ii\) a change in the membrane properties (e.g., a change in the surface density of charges or a change in the fatty acid composition of the membrane). A region of the BetP protein known as the C-terminal domain faces the cytoplasm and is mainly involved in sensing and regulating hyperosmotic stress (3). Reducing the size of the C-terminal domain by 45 amino acids (aa) resulted in BetP deregulation, while reducing the size of another region, the N-terminal domain, shifted the activity of the protein to a change upon Na\(^+\), K\(^+\), and betaine binding. K\(^+\) significantly strengthened all interactions, modulated dynamic properties of functionally important structural regions, and increased the mechanical rigidity of the protein. These changes modulating the properties of the transporter sets BetP into an active state. The binding of the substrate betaine could influence but not establish most of these K\(^+\)-dependent interactions. A pronounced conformational change triggered by K\(^+\) was observed in a particular region, the periplasmic partly helical loop9 that connects the central iris-shaped bundle helices to a scaffold of two long tilted helices. In the leucine transporter, LeuT, a similar helical segment contributes to a second substrate-binding (S2) site in the periplasm (4). The functional effect of K\(^+\) on BetP structure and dynamics, we were able to determine a mechanism by which K\(^+\) influences the interactions in and between protomers in the trimeric BetP.

In the past, a method known as single-molecule force spectroscopy (SMFS) was applied successfully to several types of transmembrane proteins to quantify their interactions and understand them at different structural levels. Another method known as dynamic SMFS (DFS) can be used to intimately investigate these interactions and reveal their dynamics in the form of kinetic parameters. Using both techniques, we structurally localized and quantified how the interactions, the protein dynamics, and the mechanical properties of the structural regions of BetP were influenced by K\(^+\).

Author contributions: C.Z. and D.J.M. designed research; L.G., C.P., and I.W. performed research; L.G., C.P., I.W., and C.Z. contributed new reagents/analytic tools; L.G., C.P., C.Z., and D.J.M. analyzed data; and C.Z. and D.J.M. wrote the paper.

The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

1L.G. and C.P. contributed equally to this work.

2To whom correspondence may be addressed. E-mail: daniel.mueller@bsse.ethz.ch or christine.ziegler@mpibp-frankfurt.mpg.de.

See full research article on page E890 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1109597108.
role of this S2 site in secondary transporters sharing a similar structural fold as LeuT has been controversial because of a limited amount of data (5). For BetP, additional experiments revealed that K⁺ triggers the formation of a second substrate-binding site. This second site shows a lower affinity for betaine when compared to the central site. Hence, the stimulating regulatory interaction between the last transmembrane α-helix (TM12) in BetP and its K⁺-sensing C-terminal domain is transmitted via transmembrane helix 12 and loop9 to form the periplasmic S2 site that might be involved in K⁺-dependent activation of BetP (Fig. P1). The data presented here contribute to the understanding of how an increase in cytoplasmic K⁺-concentrations can promote the formation of a S2 site and point towards a K⁺-driven gating mechanism as a crucial part in transport regulation. Further, these results contribute to the general understanding of secondary transport proposing a regulatory role of the second periplasmic binding site.