important transporter families. The recurrence of this structural scaffold among
the LeuT Fold transporters has stimulated conjectures of a unified mechanism
of alternating access despite significant diversity in sequence, function, and ion
coupling stoichiometries. Mhp1, a Na⁺-coupled symporter of the Nucleobase:
Cation Symporter 1 (NCS1) family, was the first LeuT Fold member to be
structurally characterized by a full complement of canonical states including
outward-facing, inward-facing, and occluded conformations, which implied a
rocking bundle mechanism. Here we report an investigation of Mhp1 dynamics
by electron paramagnetic resonance (EPR) spectroscopy. In this analysis, dou-
ble-electron-electron resonance (DEER) distance distributions between pairs of
spin labels monitored conformational changes in response to ligand-binding.
These experimental distance distributions were directly compared with the
Mhp1 crystal structures by applying MD simulations of spin label rotamers
at the double mutant sites and simulating distance histograms. The results of
this investigation support the assertion that the crystallographically-captured
conformations of Mhp1 are reflected in solution as major conformations and
that Mhp1 operates primarily through a rocking bundle-like mechanism. How-
ever, unlike LeuT, Na⁺ binding does not induce transitions between inward-
and outward-facing conformations, rather it appears that Na⁺ powers transport
exclusively through modulation of substrate binding affinity. The model of
Mhp1 transport proposed here depends critically on the description of confor-
national equilibria, as the Mhp1 transport cycle progresses through states using
low pNa to power the transport. A comparison of LeuT and Mhp1 mechanisms highlight significant mechanistic divergence that we speculate
may in part be explained by the differential mechanisms of coupling to the
Na⁺ gradient.

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A Key Role for TM5 in the Na⁺-Coupled Alternating-Access Mechanism
Revealed by Computational Analysis of the MhsT Structures
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Mammalian neurotransmitter:sodium symporters (NSSs) terminate neurotrans-
movement by the reuptake of neurotransmitters released into the synaptic cleft,
and are important therapeutic targets. Crystal structures of LeuT, a prokaryotic
NSS homolog with 12 transmembrane segments (TM5s) from Aquifex aeolicus,
reveal a structural fold characterized by two five-TM pseudo repeats, and pro-
vide the basis for mechanistic studies of the transport process driven by the Na⁺
gradient across the membrane. Two crystal structures of another prokaryotic
NSS homolog with 11 TM5s, the multi-hydrophobic amino acid transporter
(MhsTs) from Bacillus halodurans were resolved recently (Malinauskaitė et
al., in press). The substrate L-tryptophan and two Na⁺ are bound in the central
substrate binding (S1) site and the nearby Na1 and Na2 sites, respectively,
similar to LeuT. In these two MhsT structures, however, the extracellular vestibule is collapsed and the intracellular portion of TM5
(TM5i) is in either an unwound or a helical conformation.
We investigated the potential involvement of TM5i in Na2 binding and unbind-
ing by carrying out comparative molecular dynamics simulations of the models
derived from the two MhsTs structures. We find that the helical TM5i conforma-
tion is associated with a higher tendency for Na2 release. By using comparative
network analysis, we also identify allosteric pathways connecting the region
near TM5i and the Na2 binding site to the extracellular vestibule in MhsT.
Based on these results, we propose that TM5i plays a key role in the conforma-
tional transition toward the inward-open state and eventual release of substrate,
by coordinating the intracellular exit of Na2 with the rearrangement of
TM1i and the N terminus. Such a role of TM5i may be shared across the
NSS family notwithstanding the structural differences between the eukaryotic
and prokaryotic NSSs.