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Structural insights into the elevator-like mechanism of the sodium/citrate symporter CitS

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The 2-HCT family of transporters generally translocate molecules with a 2-hydroxycarboxylate motif (HO-CR1R2-COO⁻), such as citrate, malate and lactate across the plasma membrane, and activity is tightly coupled to energy from a sodium or proton gradient. *Klebsiella pneumoniae* CitS (*KpCitS*) is the best-characterized model system, which has been purified in detergent and characterized in a reconstituted state. It plays a key role for citrate uptake to ultimately produce ATP in anaerobic fermentative process. Single-molecule fluorescence spectroscopy study provided an evidence for formation of homodimeric *KpCitS*. Analysis of hydropathy profiles and rich biochemical data suggested that it consists of 11 transmembrane helices with two putative reentrant loops. Mutational studies showed that R428, which is strictly conserved in transporters of the 2-HCT family, is critical for interaction with one of the carboxylate groups of citrate. Analysis of data from kinetics experiments demonstrated that *KpCitS* carries citrate followed by binding of sodium ion. However, there are conflicting data regarding exact stoichiometry. The structure of *KpCitS* was studied extensively by electron crystallography, providing a glimpse of its global structure. The crystal structure of a homologous symporter from *Salmonella enterica* (SeCitS) recently revealed that it forms an asymmetric dimer, and that each protomer embeds a substrate translocation pathway at the interface between the transport and the dimerization domains. That structure provided the first high resolution view of a member of the 2-HCT family; however, many details in the transport cycle remained unanswered.

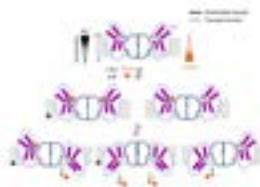


Figure1: Proposed transport mechanism of the CitS. Dimerization and transport domains are shown in dark and pale blue, respectively. The helical hairpins, HP1 and HP2, of the transport domain are represented as cylinders and loops in purple. Citrate is shown as an orange diamond, and sodium ions as black spheres. In the apo state, the binding site of substrate and ions is open toward the external environment. Binding of sodium ions prepares the protein to interact with its substrate, which promotes the elevator-like movement of the transport domain in either one of two protomers in the dimer or both. Dissociation of citrate and ions into the cytoplasm resets the protein into the outward-facing apo state. The crystal structures of CitS that have been determined by us and others²² are marked with black stars.

Biography

Subin Kim graduated from Chonnam National University in 2014 and completed her MS from Gwangju Institute of Science and Technology (GIST) in 2016 and she joined as PhD candidate in School of Life Sciences at Gwangju Institute of Science and Technology (GIST) under Mi Sun Jin in 2016.

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