MS6. Membrane proteins and signal transduction pathways

Chairs: E. Yvonne Jones, Werner Kühlbrandt

MS6-O1 High-resolution structure and substrate and ion translocation mechanism of a di-carboxylate transporter

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Membrane proteins are essential for transporting molecules across biological membranes, which makes them important drug targets. Secondary transporters, found in all kingdoms of life, transport substrates and ions across membranes and are therefore essential for many fundamental physiological processes. We have determined the crystal structure of a di-carboxylate transporter at 2.5 Å resolution to elucidate the transport mechanism. Uniquely, two different CitS homodimers are present simultaneously in the structure. In each dimer, one protomer is in the inward-facing and the other in the outward-facing conformation. Transport kinetics were determined by detailed substrate uptake measurements. Together, our data provide a complete six-step mechanism, which explains how the transporter binds the diracboxylate and substrate ions, translocates them across the membrane and the sequence in which they are released to the cytoplasm. Similar transport mechanisms may apply to a wide variety of related and unrelated secondary transporters.

Keywords: Membrane protein, X-ray structure, transporter, transport mechanism

MS6-O2 Structure, function, and inhibitors of the acid-gated *Helicobacter pylori* urea channel, an essential component for acid survival and chronic infectio

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Half the world's population is chronically infected with *Helicobacter pylori*, causing gastritis, gastric ulcers and an increased incidence of gastric adenocarcinoma. Its proton-gated innermembrane urea channel, *Hp*UreI, is essential for survival in the acidic environment of the stomach. The channel is closed at neutral pH and opens at acidic pH to allow the rapid access of urea to cytoplasmic urease. Urease produces NH_3 and CO_2, neutralizing entering protons and thus buffering the periplasm to a pH of roughly 6.1 even in gastric juice at a pH below 2.0. Here we report the structure of *Hp*UreI, revealing six protomers assembled in a hexameric ring surrounding a central bilayer plug of ordered lipids. Each protomer encloses a channel formed by a twisted bundle of six transmembrane helices. The bundle defines a previously unobserved fold comprising a two-helix hairpin motif repeated three times around the central axis of the channel, without the inverted repeat of mammalian-type urea transporters. Both the channel and the protomer interface contain residues conserved in the AmiS/UreI superfamily, suggesting the preservation of channel architecture and oligomeric state in this superfamily. Predominantly aromatic or aliphatic side chains line the entire channel and define two consecutive constriction sites in the middle of the channel. Mutation of Trp153 in the cytoplasmic constriction site to Ala or Phe decreases the selectivity for urea in comparison with thiourea, suggesting that solute interaction with Trp153 contributes specificity. The previously unobserved hexameric channel structure described here provides a new model for the permeation of urea and other small amide solutes in prokaryotes and archaea. Follow-up microsecond-scale unrestrained molecular dynamics studies now provide a detailed mechanism of urea and water transport by *Hp*UreI.

References

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