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Structure of the Na^+/H^+ antiporter NhaA

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Control by Na^+/H^+ antiporters of sodium/proton concentration and cell volume is crucial for the viability of all cells. Adaptation to high salinity and/or extreme pH in plants and bacteria or in human heart muscles requires the action of Na^+/H^+ antiporters. Their activity is tightly controlled by pH. We determined the first crystal structure of a member of this family of secondary transporters, namely the structure of NhaA [1]. The latter is the main antiporter of *Escherichia coli* and many enterobacteria. An iterative process of optimizing protein and crystal quality was pursued to permit structure determination. The protein has been crystallized at low pH, i.e. NhaA is present in its pH down-regulated conformation. A negatively charged ion funnel opens to the cytoplasm and ends in the middle of the membrane at the putative ion-binding site. There, a unique assembly of two pairs of short helices connected by crossed, extended chains creates a balanced electrostatic environment. Based on the structure and on previous biochemical and genetic data, a working model for the pH-regulated ion translocation has been suggested. Binding of charged substrates causes electric imbalance inducing movements, which allow for a rapid alternating access mechanism. This ion exchange machinery is regulated by a conformational change elicited by a pH signal perceived at the cytoplasmic funnel entry.

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Structures and Mechanisms of Human Oxygenases

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In metazoans, the cellular response to low pO_2 is mediated by the activation of the hypoxia-inducible factor (HIF) transcription factor, which initiates the transcription of an array of genes such as EPO and VEGF. HIF is active as an α/β heterodimer, however, during normoxia the α-subunit (HIF-α) is hydroxylated by three HIF prolyl hydroxylase isozymes (PHD1, 2, and 3) and an asparginyl hydroxylase, factor inhibiting hypoxia (FIH). Hydroxylation of either of 2 prolines within the oxygen dependent degradation domain of HIF-α promotes its interaction with the von Hippel-Lindau protein and subsequent ubiquitylation and proteasomal destruction whereas hydroxylation of an asparagine within the C-terminal transactivation domain prevents the interaction between HIF and the transcriptional co-activator p300/CBP resulting in its decreased transcriptional efficacy [1]. HIF hydroxylases are members of the 2-oxoglutarate (2OG) dependent iron oxygenases. Inhibition of the HIF hydroxylases in cells mimics hypoxia and results in the upregulation of HIF target genes, thus is of pharmaceutical interest. Crystallographic studies on the HIF hydroxylases [2,3] and other human 2OG oxygenases [4] will be described and discussed in the context of the role of the enzymes as oxygen sensors and in lipid metabolism.

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