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Crystal structure reveals the mechanism for highly specific interaction between CHP and NHE family and its role in pH regulation

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The Na⁺/H⁺ exchanger NHE is an integral membrane protein which catalyzes Na⁺ influx coupled with H⁺ efflux across the plasma membrane and other intracellular organellar membranes and plays a major role in maintaining ionic homeostasis such as pH_i, cell volume and transepithelial Na⁺ absorption. Calcineurin B homologous protein (CHP) is an essential cofactor that regulates the plasma membrane NHEs through interaction with the cytosolic domain. We solved the crystal structure of CHP (CHP2 isoform) complexed with its binding domain in NHE (NHE1 isoform) at 2.7 Å. The structure shows that CHP-binding region is an α-helix which is tightly inserted into the hydrophobic cleft of CHP2. This cleft constitutes the region that traps the cytoplasmic α-helix of NHE1 and maintains the stability of the complex by means of hydrophobic interactions. A large contact area of about 1466 Å² exists between CHP2 and NHE1-peptide reflecting the tight interaction between the two molecules. Interestingly, target peptide shows a uniform distribution of hydrophobic residues all facing toward the cleft of CHP2, making the binding of CHP2 to NHE1 very specific. Furthermore, the crystal structure of CHP2 revealed the presence of a protruding region connecting both the N- and C-lobes. Deletion of this CHP-unique region significantly reduced the physiological exchange activity by inducing an acidic shift of the pH_i dependence, while preserving the interaction with NHE1, suggesting that this region functions as a critical segment controlling pH_i regulation by NHE1.

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Normal Mode Analysis of a Small Membrane Polypeptide

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Recent progress in X-ray crystallography and NMR techniques has led to the determination of three-dimensional structures of many biological molecules. In the post-genome era, it is a challenging problem to identify the relationship between structures and functions of biomolecules. Polytheonamides are highly cytotoxic polypeptides isolated from the marine sponge *Theonella swinhoei* in 1994. The 2D-NMR analysis indicated that polytheonamide B is a linear 48-residue peptide with alternating D- and L-amino acids and contains seven kinds of methylated and hydroxy variants of proteinogenic amino acids [1]. To investigate the dynamical properties of polytheonamide B we performed energy minimization and normal mode analysis by using the program CHARMM. We calculated root-mean-square displacements of backbone atoms, root-mean-square fluctuations of the backbone dihedral angles (ϕ , ψ), and correlation factors for the dihedral angle fluctuations. The normal mode analysis revealed that polytheonamide B shows the elastic rod behavior in the low frequency modes and that the libration motions of backbone amide planes, which would be relevant to the function of polytheonamide B, have the modes with the frequencies in the region of 50-180 cm⁻¹. We compare our results with those of the studies on another D, L-alternating polypeptide.

[1] T. Hamada, S. Matsunaga, G. Yano, and N. Fusetani, *J. Am. Chem. Soc.*, **127**, 110-118 (2005).

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Single-molecular gating dynamics of KcsA potassium channel measured by diffracted X-ray tracking

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Crystal structure of several types of potassium channels has revealed closed and open conformations of the gate. To detect the movement of the gate in real time, we adopted diffracted X-ray tracking (DXT) method to the KcsA potassium channel. The purified channel was fixed in upright position on a thin glass plate. One-dimensional gold nano-crystal was attached to the KcsA channel through introduced cysteine residues. Synchrotron X-ray beam was radiated normal to the glass plate. In this geometrical configuration, diffraction spot moved radially when the channel tilted, the spot moved circumferentially when the channel rotated. To detect motion of the gate, the cytoplasmic domain was deleted and the C-terminal end of the M2 helix was labeled by the nano-crystal. At pH 7.5 where the channel is closed, DXT revealed small tilt motion. On the other hand, at pH 4.0 where the channel is actively gated, rotational movement was detected. It means a bend of the M2 helix upon opening lead to the rotational motion of the nano-crystal. When the cytoplasmic domain was intact and labeled at the C-terminal end, the rotational motion with more tilt motion was detected. These results indicate that the gating motion is spread to the end of the cytoplasmic domain.

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Structural Analysis of Non-Selective Cation Channel TRPV4 using a Phase-Contrast Transmission Electron Microscope

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Transient receptor potential (TRP) family is widely expanding superfamily of non-selective cation channels; most of them are permeable for Ca²⁺. TRP superfamily is classified into seven subfamilies according to the sequence homology: the TRPC (Canonical) family, the TRPV (Vanilloid) family, the TRPM (Melastatin) family, the TRPP (Polycystin) family, the TRPML (Mucolipin) family, the TRPA (Ankyrin) family, and the TRPN (NOMPC) family. Some of TRPV and TRPM are known to as a temperature-sensitive channel, although each has a different range of temperature sensitivity. TRPV4 is activated by hypotonic stimuli and also by warm temperature; ~27-35°C resulting in an increase in intracellular Ca²⁺ concentration. All TRP channels have putative six-transmembrane (6TM) domains, which are thought to assemble as tetramer to form cation-permeable pores. To dissolve the tertiary structure of such macromolecules, transmission electron microscopy (TEM) is known as one of powerful methods. Recombinant TRPV4 was expressed using baculo virus-infected *Sf-9* cells and purified using genetically attached hexa histidine-tag. The membrane fraction was solubilized by detergent; DDM (n-Dodecyl β-D-maltoside) and purified by affinity chromatography and following size-exclusion chromatography. The detergent solubilized TRPV4 was analyzed using a phase-contrast cryo-TEM in its size distribution and purity. The single-particle analysis of its tertiary structure has been underway and the preliminary structure has been determined.