IP110 ウシ心筋 NADH-ユビキノン酸化還元酵素の 2 次元結晶化
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2D-crystallization of bovine heart NADH-ubiquinone oxidoreductase.
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NADH-ubiquinone oxidoreductase (Complex I) is a main entry point of mitochondrial and bacterial respiratory chains. Bovine heart Complex I is one of the largest known membrane protein complexes with molecular mass of about 1,000 kDa, containing at least 45 different subunits. The enzyme transfers electrons from NADH to ubiquinone, coupled to proton pumping. The protein has characteristic L-shaped structure. One arm is embedded in the membrane and the other, peripheral arm, protrudes into the mitochondrial matrix. It is well known that the native enzyme is highly sensitive to its inhibitors, rotenone and piericidin A. The atomic structure of the complete Complex I is not yet known, although the hydrophobic domain of a bacterial Complex I with the molecular mass of 550 kDa has been reported at 3.3 Å resolution (Sazanov, L. A. and Hinchliffe, P. Science, 311 (2006) 1430-1436). Recently, we have significantly improved the purification method of bovine heart Complex I and found that the improved enzyme preparation was significantly stabilized by addition of phospholipids. Extensive survey in terms of phospholipids species, dialysis procedure and other various factors provided 2D-crystals of the enzyme whose negative-staining images showed optical diffraction patterns. No significant difference was detectable between SDS-PAGE pattern of the 2D crystals and that of the preparation before crystallization, suggesting that 2D-crystals of the complete bovine heart enzyme were obtained.

IP112 SDSL-ESR 法による Na⁺/H⁺交換輸送担体 (NhaA) の基質結合による構造変化の検出
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Detection of substrate induced conformational changes of Na⁺/H⁺ antiporter (NhaA) by
SDSL-ESR
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ite-directed spin-labeling electron spin resonance (SDSL-ESR) spectroscopy has been applied for determining secondary, tertiary and quaternary structures, and associated conformational changes of proteins. Depending on the rigidity of attached spin label, it is possible to investigate steric restrictions imposed by the environment of the label or motion of a segment of a protein. In this study, we focused on the ion transporting membrane protein, Na⁺/H⁺ antiporter (NhaA), and discussed the conformational changes during ion transport. NhaA is the major transporter in Na⁺ efflux system in bacteria. And NhaA accomplishes specific exchange of Na⁺ or Li⁺ for H⁺. Crystallographic studies showed that NhaA has negatively charged funnels in the hydrophobic lipid bilayer. The funnels could be the transport pathway, in which substrate binding sites are located. In alternating access model, transporters and also antiporters may transport substrates by conformational changes causing the topological changes of funnels. We measured ESR spectra from spin-labels on introduced Cys residues of a middle of transmembrane helices which face the funnel. Some of ESR spectra showed their spin-label mobility were changed by pH change between 4 and 7. This result suggested that H⁺ induced conformational changes occur in a region closed to substrate binding site.

IP111 ブタ腎 Na⁺/K⁺-ATPase オリゴマーの電子顕微鏡観察
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Electron microscopic study of pig kidney Na⁺/K⁺-ATPase protomers in oligomeric form
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Detergent C₁₂E₈ solubilized Na⁺/K⁺-ATPase from pig kidney was separated into three major protein peaks according to their retention times by gel filtration chromatography using modified Shimadzu HPLC system. These three peaks had been established to contain solely protomer (P: αβ), dimer (D: (αβ)₂) and tetramer (T: (αβ)₄) based on their estimated molecular weight by using low-angle laser light scattering measurement (Hayashi et al., *BBA*, 1989. and Hayashi et al., in: *Na,K-ATPase and Related Cation Pumps*, 2002). The oligomer species were then frozen-dried and shadow-cast with Pt/C, and observed using a normal electron microscope. Micrographs contained mainly three different sizes of particles, S, M and L in the order of their size. The ratio of numbers of S×1, M×2 and L×4 showed very good accordance with the ratio of peak area size of P, D and T. This indicated that the S, M and L particles were P, D and T, respectively. The image of T showed roughly a rectangular shape which was also observed in frozen-hydrated specimen using an electron cryo-microscope. We concluded that the oligomer formation was a nature of Na⁺/K⁺-ATPase and played some regulatory or functional role in the cation transport process coupled with ATP hydrolysis.

IP113 高親和性 IgE 受容体 β 鎖 ITAM モチーフの構造解析
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Structural study of mutated FcεRI β chain ITAM by Far-UV and Near-UV CD
spectroscopy.
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Upon FcεRI stimulation, mast cells secrete inflammatory cytokines and chemical mediators are related to allergic inflammation. FcεRI complex shows a tetrameric structure composed of three distinct polypeptides, which include the IgE-binding α chain, the four-folded membrane-spanning β chain, and the disulfide-linked γ-γ homodimer. The β chain acts as an amplifier for allergic reaction in mast cells. The β chain ITAM shows a notable departure from the consensus immunoreceptor tyrosine-based activation motif (ITAM) sequence with the presence of the third tyrosine between the two canonical tyrosines (YX₂YX₃Y). Our previous study revealed that degranulation and leukotriene production were impaired in cells expressing the mutated FcεRI β ITAM (FFF,FYF,FYY, YYF). However, cytokine production was enhanced in the YFY and FFF mutant. Here, we describe conformational studies of the C-terminal 43 peptide of wild type and mutated FcεRI β ITAM motifs FFF,FYF,YFY,FYY and YYF by CD spectroscopy. All of the far-UV CD spectra of WT, FFF, FYF, YFY, FYY, and YYF give typical α-helical feature. The temperature dependence of CD signal showed that midpoint temperature (T_m) decreased by almost 2 degree in FFF and YYF compared to that in WT.