2P312 ホスホリパーゼC-d1 PH ドメインおよびEF-hand ドメインの脂質二重膜上における構造とダイナミクス
○三宅 健史¹、川合 克久¹、八木澤 仁¹、辻 暁¹
2pK06 ¹兵庫県立大・院生命理
Structure and dynamics of phospholipase C-d1 PH- and EF-hand domain at the membrane surface
Takeshi Miyake(1), Katsuhisa Kawai(1), Hitoshi Yagisawa(1), Satoru tuzi(1).(1: Grad. Schl. Life Sci Univ. hyogo)

Phospholipase C-d1 (PLC-d1) is included in the cellular signal transduction pathway, and hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) to provide inositol 1,4,5-trisphosphate (IP3) and diacylglycerol as the second messengers. In the previous works, we have reported characteristic conformational changes of the isolated N-terminal PH domain of PLC-d1 induced at the lipid bilayer surface by using solid state NMR. Since the PH domain is known to dominate membrane localization of PLC-d1 through its specific binding affinity to PIP2, those conformational changes are expected to contribute to the regulation of the structure and function of PLC-d1 at the membrane surface. In this work, we focused on the structure and dynamics of membrane bound PLC-d1 fragment (1-224) containing the PH- and EF-hand domains. Solid state ¹³C NMR spectra of [3-¹³C]Ala labeled PH domain-EF-hand domain fragment (1-224) containing the PH domain and N-terminal two EF-hand motifs indicated a conformational alteration of the long amphipathic loop (b5/b6 loop) of the PH domain compared with the isolated PH domain (1-140) either in solution or at the membrane surface. Although ¹³C NMR signals of the EF-hand region (141-224) revealed no stable secondary structure, this region of the EF-hand domain would cause the conformational change of the PH domain through the direct inter-domain interaction and/or a modification of the property of the lipid bilayer. Observed dynamics of the lipid bilayer in the presence of the fragment suggests contribution of the latter.

2P314 光化学系 II における水分解・酸素発生反応の新しい酵素触媒機構
○楠 正美¹
2aD03 ¹明治大理工物理
New enzyme catalysis mechanism of the water-splitting and oxygen-evolving reaction in photosystem II
Masami Kusunoki. (Dept Physics, School of Sci & Techn, Meiji Univ.)

The molecular mechanism of the water-splitting and oxygen-evolving reaction in photosystem II (PSII) has been a subject of considerable studies since B. Kok proposed a phenomenological linear four-step mechanism (1970). This reaction takes place in an oxygen evolving complex (OEC), which incorporates four Mn, one Ca and one Cl ions, to cycle five intermediates, S_i (i=0-4). Recent remarkable improvements in X-ray diffraction data of PSII established the existence of a catalytic Mn₄Ca cluster ligated by six protein carboxylates and one His, which could be used to narrow down possible structures of the Mn₄Ca cluster. After this, we presented a chemically complete model for the Mn₄Ca cluster in S₀-state with its surrounding enzyme field (involving Cl⁻ and H337), in which the terminal Mn ion is mono-μ-oxo-mono-μ-carboxylato bridged to the compact Mn₃CaO₃(OH) subunit and binds two substrate water molecules at the cis-positions. We could show that successive oxidations of this cluster lead to the most probable tautomer at each S_i-state (i=1-3) whose structure can reasonably well explain key experimental data, not only from EPR and (Mn²⁺ and Ca²⁺-K-edge) EXAFS spectroscopy but also from the S_i-state dependence of ¹⁸O exchange rates of two substrate water molecules, using the DFT/B3LYP method to confirm the best fitting with the 3.0 Å resolution Berlin model too. Moreover, we will present a new mechanism of the O-O bond formation which can take place in the correlated transition states of two substrate exchange processes at the terminal Mn ion.

2P313 細胞内での上皮成長因子受容体とアダプター分子間解離速度の計測
○森松 美紀¹、松田 道行¹
¹京大院・生命科学・生体制御
Efficient method for measuring dissociation rates between epidermal growth factor receptor and adaptor proteins in living cells
Miki Morimatsu (1) and Michiyuki Matsuda (1) (1: Laboratory of Bioimaging and Cell Signaling, Graduate School of Biostudies, Kyoto Univ.)

Epidermal growth factor receptor (EGFR) causes autophosphorylation upon stimulation of EGF and many signaling molecules bind to the phosphorylated tyrosine residues on EGFR to transmit the external signals into the cell. In order to understand the EGF-signaling mechanism quantitatively, the information of reaction kinetics is indispensable. Although the reaction rates have been determined using EGFR peptide in *vitro*, the reaction rates between intact molecules in living cells are yet to be obtained. In this study, we established a method for measuring dissociation rates between EGFR and adaptor proteins using confocal microscopy. EGF stimulation triggers EGFR endocytosis within several minutes and adaptor proteins are located on endosome with the phosphorylated EGFR, repeating a cycle of association and dissociation. Shc is one of the adaptor proteins that bind to the phosphorylated EGFR. To measure the dissociation rate of Shc from the phosphorylated EGFR, PA-GFP (photoactivatable GFP) fused to the C-terminus of Shc was expressed in A431 cells. Endosome was irradiated by 405 nm laser to photoactivate PA-GFP and the decay of PA-GFP fluorescence intensity on the endosome was observed. The decay curve was fitted to an exponential function and dissociation rate of 0.58/s was obtained. This method takes advantage of the endosomes containing dominantly phosphorylated EGFR and allows us to measure the dissociation rate of various EGFR-binding proteins with high signal to noise ratio.

2P315 光合成光化学系 II において蛋白質ゆらぎはP680を還元するプロトン共役電子移動で重要な役割を演ずる
○斉藤 圭亮¹、向井 宏一郎¹、住 斉¹
2aD02 ¹筑波大・院・数理物質
Important role of protein matrix fluctuations in the proton-coupled electron transfer of the reduction of P680 in photosystem II
Keisuke Saito (1), Koichiro Mukai (1), Hitoshi Sumi (1). (1: Inst of Materials Science, Univ of Tsukuba)

The reaction center of photosystem II in photosynthesis photo-oxidizes water. This oxidation starts from formation of oxidized P680 (P⁺), a central chlorophyll pair, by light-induced charge separation. After the charge separation, P⁺ is reduced by an adjacent tyrosine residue of Yz. In association with the reduction, a proton on Yz is transferred to an adjacent histidine residue in the protein matrix. Here, the electron transfer and the proton transfer are coupled. Concerning this coupled reaction, it has been debated which of the proton or the electron is transferred first otherwise that they are transferred concertedly. We describe such a proton-coupled electron transfer theoretically. The wavefunction of the proton is so localized that the overlap between wavefunctions, which is generally most important in determining to the rate constant, is sensitive to the environment change in comparison with in the electron transfer. In the proton transfer, therefore, slow fluctuations of the protein matrix inducing the distance change between proton pockets play an important role. We take into account the effect of such fluctuations. We show theoretically that the electron and the proton are transferred concertedly in the reduction of P680 in photosystem II. Furthermore, it has been observed that the rate constant has a magnitude of order 0.1 ns⁻¹ and the isotope effect between the proton and the deuteron transfer is very small at 2-3. We also show that these observations are rationalized only by the effect of such slow fluctuations.