which are characterized as the neuropathological hallmarks of Alzheimer's disease. Recently, it was revealed that a peptidyl-prolyl isomerase Pin1 restored the ability of phosphorylated tau protein to bind tubulin and promote microtubule assembly. Pin1 specifically isomerizes phosphorylated serine or threonine that precedes proline (pS/T-P) and regulates the function of the phosphoproteins. The mechanism of regulation of tau protein by Pin1, however, is still unsolved. Here we investigated interactions between tau protein and Pin1 by surface plasmon resonance and fluorescence anisotropy. As a result, Pin1 interacted with all the pS/T-P sites of tau protein, but their interactions were too weak and transient to form a stable complex. This suggests that Pin1 may interact with tau protein at another site to restore the activity of tau protein. So we synthesized various peptides covering the whole sequence of tau protein, and investigated the interactions between tau protein and Pin1. This result will be discussed at this annual meeting.

3C0948 ヒトガレクチン1糖結合能のpH依存性とヒスチジンの役割

Involvement of Histidine Residues in the pH-dependent β -Galactoside-binding Activity of Human Galectin-1

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Human galectin-1 (hGal-1) is an animal lectin that shows affinity for β -galactosides. The binding of hGal-1 to glycoproteins and/or glycolipids regulates various physiological phenomena. It is known that interactions such as hydrogen bonding and CH- π interaction are important in the lactose (Gal β 1-4Glc) binding of hGal-1 and the lactose-binding constant (Kb) of hGal-1 depends on pH. The pH dependence may be related to the role of hGal-1 in vivo, where pH varies temporally and locally. We investigated the origin of the pH dependence of Kb by using fluorescence, circular dichroism, UV absorption, and UV resonance Raman spectroscopy. Kb of wild-type hGal-1 decreased below pH 6.0, and the pKa values of the His44 and His52 side chains were determined to be 5.7 and 6.2, respectively. Mutation of one of the His residues to Gln decreased the Kb value to 0 and 40% of the wild-type for His44Gln and His52Gln, respectively. These results suggest an involvement of the His residues in the pH dependence of Kb. Results of spectroscopic measurements suggested the occurrence of cation- π interaction between the protonated His44 and Trp68 and of an electrostatic interaction between the protonated His52 and a neighboring residue in the lactose-free form at acidic pH. The interactions of protonated His residues may disfavor the interactions between hGal-1 and lactose that are crucial for the lactose binding. The interference of the lactose-protein interactions by protonated His residues may explain the decrease of Kb at acidic pH.

3C1012 枯草菌 ferredoxin-NADPH 酸化還元酵素の C 末端サブドメインの機能

Functions of a C-terminal subdomain in the ferredoxin-NADPH oxidoreductase from the bacterium *Bacillus subtilis*

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The gram-positive bacterium *Bacillus subtilis* contains a novel type of ferredoxin-NAD (P) H oxidoreductase (FNR) with high homology to the NADPH-thioredoxin reductase (Tdr) in amino acid sequence level. Recently, we have determined the crystal structures of *B. subtilis* FNR (BsFNR) by X-ray crystallography. BsFNR retains its structural topology with *E. coli* Tdr but an additional C-terminal sub-domain covers the re-face of the isoalloxazine ring of FAD prosthetic group in BsFNR. The sub-domain includes the aromatic residue stacked on the re-face of the isoalloxazine ring of FAD prosthetic group. On the si-face of the ring, Tyr residue stacked on. These two aromatic rings are positioned almost parallel with respect to the isoalloxazine ring at distances of 3.5 Å. Such a configuration of the rings is absent from Tdr but found in plastid-type FNRs, suggesting that this structural character is indispensable for the FNR reaction. To elucidate the functional details of the subdomain and the residues involved in the subdomain, steady state reaction analysis of deletion mutants was performed. Obtained results suggest that the subdomain perturb an efficient hydride ion transfer with NADPH but diminishes an electron escape to non-physiological electron acceptors. Effects of deletions on the reactions with Fd will be also discussed.

3C1024 分子動力学計算による GluR2 のリガンド結合部位におけるグルタミン酸結合およびクレフト閉口過程の解析

Molecular dynamics simulations for glutamate-binding and cleft-closing processes of the ligand-binding domain of GluR2

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Ligand-gated ion channels mediate synaptic signals in the central nervous system. X-ray crystal structural analyses have revealed that the gating of the ion channels of ionotropic glutamate receptors is controlled by the cleft-closure of the ligand-binding domain (LBD) of GluR2 induced by the glutamate binding. Because it is difficult to trace the movement of the glutamate by experiments, we examined the roles of residues of the LBD in the glutamate-binding and cleft-closing processes by spontaneous molecular dynamics (MD) simulations. A glutamate entered the cleft deeply within the order of nanoseconds under the influence of electrostatic attraction by the LBD. The role of TYR450 locating at the center of the cleft is speculated to be guiding the glutamate upon binding by the cation- π attractive interaction. The cleft locked the glutamate completely at 15 ns in an MD run, and the obtained binding mode is the same as that observed in the X-ray co-crystal structure. We calculated the potential of mean force by using the structures on the glutamate-binding and cleft-closing pathway. The free energy profile showed that there is a semi-open state between two transition states. GLU705 in the so-called sub-domain 2 forms a hydrogen-bond bridge with TYR732 in the sub-domain 1, presumably reinforcing the semi-open structure for the transition occurring before the closed state.

3C1036 Nitric Oxide Is Formed By Reacting Nitrite Ion and Deoxyhemoglobin: New Insights

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Naturally occurring nitric oxide (NO) –synthesized by specific nitric oxide synthases—is responsible for the vasodilation of smooth muscle. In recent years, however, a new possible NO regeneration pathway has been reported from the reaction between nitrite –a natural byproduct of the NO action–and deoxyhemoglobin (deoxyHb).

We have investigated the effects of pH, the stabilization of the R-quaternary conformation of deoxyHb by the enzymatic removal of the residues Tyr145 and His146 from the β -subunits of Hb (desHisTyr-Hb), and the blocking of the sulfhydryl group of Cys β 93 with iodoacetamide (AA-Hb) on the above-mentioned reaction at equimolar concentrations of heme:nitrite under anaerobic conditions at 25°C. After 12 hours, the fractions of nitrosyl-Hb (NO-Hb), oxidized Hb (metHb), and the remaining unreacted deoxyHb were estimated by deconvolution of the absorption spectra.

In all cases, the unreacted fraction of deoxyHb remained elevated at high pH, whereas metHb formation was enhanced at low pH, more remarkably in the case of Hb-AA and desHisTyr-Hb-AA (both ~80%), but not desHisTyr-Hb ($\leq$ 20%). At pH 7.4, the metHb and NO-Hb fractions remained in all cases around 50%. Locking deoxyHb in the R-conformation did not affect the NO-Hb formation. NO-Hb formation was low (~20%) at high pH. In contrast, this effect was reversed in the case of AA-Hb (40–50% NO-Hb). The specific reactivity of α and β subunits, as well as the possible participation of the sulfhydryl group at Cys β 93 are under investigation.

3C1048 一酸化窒素還元酵素における基質結合状態についての理論的研究 Theoretical investigation on the substrate binding states of nitric oxide reductase

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Nitric oxide reductase (NOR) catalyzes a reduction of nitric oxide (NO) to nitrous oxide (N₂O), which is an important step in the denitrification process of anaerobic respiration: NO₃⁻ → NO₂⁻ → NO → N₂O → N₂. In 2010, first x-ray crystal structure of NOR was reported at atomic resolution (2.7 Å) [1]. It was revealed that NOR has many structural similarities to cytochrome oxidase (COX), in consistent with the evolutionary relatedness. The reaction mechanism of NOR is, however, not fully elucidated. In this study, the first state of the reaction, substrate-binding state, was investigated by the density functional theory (DFT) calculations. Optimized structures and the substrate binding energies were examined according to proposed cis- and trans-mechanisms.

[1] T. Hino, Y. Matsumoto, S. Nagano, H. Sugimoto, Y. Fukumori, T. Murata, S. Iwata, Y. Shiro, *Science*, 330, 1666 (2010)