1P026 CD28 細胞内領域とシグナル伝達分子との相互作用解析
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Interactions of recombinant Gads, Grb2, p85 subunit of PI3K, and their SH2 domains
with CD28 cytoplasmic domains
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CD28 provides a costimulatory signal that is required for full T cell activation and expression of T cell functions. CD28 has cytoplasmic domain of 41 amino-acid residues, which contains both SH2 and SH3 binding motifs, and associates with signaling proteins such as PI3K and Grb2 family proteins, Grb2 and Gads, via their SH2 domains. These signaling proteins also have SH3 domains, and their contributions on the binding to CD28 are controversial. In this study, we overexpressed recombinant Gads, Grb2, p85 subunit of PI3K, and their SH2 domains in *E. coli*, and analyzed their interactions with the chemically synthesized wild-type and mutant CD28 cytoplasmic domains, using surface plasmon resonance biosensor, isothermal titration calorimetry, and X-ray crystallography. The association constant of Gads SH2 domain to the wild-type CD28 was similar to that of Grb2 SH2 domain, and was lower than those of p85 N- and C-terminal SH2 domains. The complex of Gads SH2 domain and the SH2 binding region of CD28 was crystallized, and its structure was determined at 2.7 Å resolution. The association constant of Grb2 to the wild-type CD28 was similar to that of the mutant CD28, in which Pro residues in the SH3 binding motifs were mutated to Ala, indicating that only the SH2 binding is critical for the Grb2 binding. Because of the aggregation property, it was difficult to purify Gads, but the addition of arginine was found to be effective. Together with the results of Gads and p85, we discuss their recognition mechanisms with CD28.

1P028 ランダム配列タンパク質からの機能と構造の進化
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Evolution of functionality and structure from a random-sequence protein.
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Natural protein sequences encode characteristic features such as functionality, foldability and thermodynamic stability absent from ordinary random sequences. Though the sequences, in any case, were fabricated by evolution under natural selection, it is not clear how the sequences had evolved in the first place. Here as a model of an early stage of the protein evolution, we have been observed an experimental evolution of random-sequence protein (about 140 residues) by exploring around a single initial sequence using random point mutagenesis and simple functional selection for DNA-binding ability. As a result of the evolution through 25 generations, functionality (DNA-binding ability) was gradually increased. By phylogenetic analysis of selected sequences, the rise and fall of several branches in the phylogenetic tree were observed. Physical properties of two proteins evolved in different branches of the phylogenetic tree at 24th and 25th generation respectively and of the initial protein were examined. All the three proteins were soluble and existed as compact monomers comparable with the size of molten globule state of natural proteins. Furthermore, the structure of the proteins was also evolved from fully unordered heterogeneous structures to more ordered flexible structures containing polyproline II helix type conformation. Notably, similar evolution was observed in other experimental evolutions starting from other initial sequences, hence such evolution is general phenomena.

1P027 マルチカノニカルシミュレーションによって描かれる、水中のアルツハイマーβアミ
ロイドペプチドAβ(12-36)の自由エネルギー地形
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Free-energy landscape of the disordered state of an Alzheimer's β amyloid peptide in
water studied by multicanonical molecular dynamics
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The monomeric Alzheimer's β amyloid peptide (Aβ) is known to adopt a disordered state in water at room temperature, and the secondary-structure contents for the disordered state had been determined by a circular dichroism (CD) spectroscopy experiment: 70% random, 25% β-structural, and 5% helical. We performed an enhanced conformational sampling (multicanonical molecular dynamics simulation) of a 25-residue segment (residue 12-36) of Aβ in explicit water to obtain a conformational ensemble equilibrated in water at 300K. The secondary-structure contents calculated from the conformational ensemble at 300K reproduced the experimental ones. The constructed free-energy landscape at 300K was not plain but rugged with five clusters, and each cluster had unique tertiary structure: a helix-structural cluster, two β-structural clusters, and two random-structural clusters. This indicates that the contribution from the five individual clusters determines the secondary-structure contents experimentally measured. The helical cluster had a similarity with a stable helical structure for monomeric Aβ in 2,2,2-trifluoroethanol (TFE)/water determined by an NMR experiment. The cluster-cluster separation in the conformational space indicates that free-energy barriers separate the clusters at 300K. The two β-structural clusters were characterized by different strand-strand hydrogen-bond (H-bond) patterns, suggesting that the free-energy barrier between the two clusters is due to the H-bond rearrangements.

1P029 脱窒菌 *Alcaligenes xylosoxidans* における亜硝酸還元酵素-シトクロム c551 間電子
伝達速度論的ならびに構造的特徴
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Kinetic and structural evidence for the electron transfer between cytochrome c551 and
nitrite reductase from *Alcaligenes xylosoxidans*
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Dissimilatory nitrite reductase (NIR) is a key enzyme in denitrification, catalyzing the first step that leads to gaseous products (NO, N₂O, and N₂). NIR from *Alcaligenes xylosoxidans* (Ar-NIR) has a trimeric structure and contains two copper centers, which are called Type 1 Cu and Type 2 Cu, respectively. Type 1 Cu accepts an electron and transfers it to type 2 Cu center. Type 2 Cu is a catalytic center for the reduction of NO₂ to NO. We have demonstrated that cytochrome c₅₅₁ (Ar-Cyt c₅₅₁) from *Alcaligenes xylosoxidans* effectively donates an electron to Ar-NIR by using electrochemical measurements. To investigate the electron transfer reaction between these proteins, stopped-flow and steady-state kinetic experiments have been carried out. The second order rate constant of the electron transfer between Ar-Cyt c₅₅₁ and Ar-NIR was 2.01(±0.16)×10⁶M⁻¹s⁻¹. In addition, we have determined the crystal structure of an electron transfer complex between these proteins at 1.7 Å resolution. The complex structure consists of one Ar-NIR trimer and one Ar-Cyt c₅₅₁ molecule. Ar-Cyt c₅₅₁ binds to the surface on the Type 1 Cu in a monomer of Ar-NIR. The distance between Type 1 Cu of Ar-NIR and heme edge of Ar-Cyt c₅₅₁ was about 10.5 Å. This result suggests the rapid electron transfer reaction observed in stopped flow experiment.