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Extracellular giant hemoglobins (Hbs) show remarkably different quaternary structures and oxygen binding properties from those of vertebrate tetrameric Hbs. Several crystal structures of 400 kDa Hbs have been determined, but no deoxygenated (fully unliganded) structure is available. To obtain detailed structural information of both the oxy and deoxy forms of the giant Hb, we have initiated crystallographic studies of 400 kDa Hb (V2Hb) from a tube worm, *Lamellibrachia satsuma*.

We have previously determined crystal structures of *Lamellibrachia* V2Hb in the oxy form. The deoxygenated crystals have been successfully prepared from the oxygenated crystals by soaking into a solution containing sodium hydrosulfite, and the structure of deoxy form has been determined at 2.9 Å resolution. The structure transitions between the oxy and deoxy forms of V2Hbs are primarily observed at AB loop regions of each globin subunits. The structural changes at the AB loops cause conformational changes of the E helices at the positions of Val E11, which are located just above the ligand-binding pockets, and thought to play a key role to regulate affinities for the ligands. These tertiary changes are influenced each other primarily by quaternary changes around the interface of the neighbouring subunit, where the each hemes locate face to face. Relatively small quaternary changes are observed around molecular 3-fold and 2-fold axes, to keep the molecular symmetry of V2Hb.

1P041 ヒトリボカリン型プロスタグランジン D 合成酵素の脂溶性低分子に対する相互作用解析

Interaction Analysis of Human Lipocalin-type Prostaglandin D Synthase for Small Lipophilic Ligands

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Lipocalin-type prostaglandin D synthase (L-PGDS) is a multi-functional protein belonging to the lipocalin family, acting as a PGD₂-producing enzyme and as a secretory transporter protein for lipophilic molecules. In the present study, we focused on the function of human L-PGDS as a transporter, and aimed to clarify the binding affinities of L-PGDS for various lipophilic molecules.

Taking advantages of the fluorescence quenching of the intrinsic tryptophan residues, we investigated the binding ability of L-PGDS for lipophilic molecules such as biliverdin (BV), all-*trans*-retinoic acid (RA), *L*-thyroxine (T4), progesterone (PS) and genistein (GS). After incubation with the ligands at 25 °C for 30 min, the fluorescence intensity of the tryptophan residues was measured with an excitation wavelength at 290 nm and an emission wavelength at 334 nm. The fluorescence intensity decreased by the addition of various ligands in a concentration-dependent manner. Based on the fluorescence quenching, the dissociation constant values of L-PGDS for BV, RA, T4, PS, and GS were calculated to be 19 nM, 170 nM, 360 nM, 2.4 μM and 5.6 μM, respectively. Further, in order to obtain more detailed information on the interaction between L-PGDS and the ligands, we have been performing isothermal titration calorimetry.

Our results indicate that human L-PGDS binds hemoglobin metabolites such as BV with significantly higher affinities than the other ligands. These results suggest that human L-PGDS acts as a scavenger for the hemoglobin metabolites.

1P042 Molecular dynamics studies on Mutational structures of a Nylon-6 Byproduct-degrading Enzyme

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We have studied catalytic mechanisms of a Nylon-6 byproduct-degrading enzyme as a model system to reveal the bio-degradation of unnatural synthetic compounds. 6-Aminohexanoate-linear dimer, which is a kind of Nylon-6 byproduct, is hydrolyzed by this enzyme according to the following reaction. $\text{H}_2\text{N}-(\text{CH}_2)_5-\text{CONH}-(\text{CH}_2)_5-\text{COOH} + \text{H}_2\text{O} \rightarrow 2 \text{H}_2\text{N}-(\text{CH}_2)_5-\text{COOH}$

For this enzyme, a lot of site-specific mutagenesis studies were performed experimentally by Negoro's group in order to understand the catalytic mechanisms and improve its catalytic activity. According to their mutagenesis works, Ser112 serves as a main catalytic residue, performing a nucleophilic attack on amide compounds. Moreover, some important residues participate in the degrading reaction. However, no experimental evidence that indicates the structure-function relationships of the mutants is provided, because the most of the mutational structures have not been determined by the X-ray experiment yet. In this work we thus determined thermal equilibrium structures of the substrate-binding complex of the wild type and the mutational enzymes

in the aqueous solution by using the molecular dynamics simulations. In our poster, we will discuss the conformational changes around catalytic sites due to the mutations and relationships between calculated structures and mutagenesis experiments.

1P043 3次元 RISM リガンドマッピング法を用いたフラグメントベースの薬物設計: FK506 結合タンパク質への応用 Fragment-based drug design using the 3D-RISM ligand-mapping method: An application to FK506-binding protein

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Fragment-based drug design (FBDD) is one of the most promising alternatives to traditional high-throughput screening approach. In FBDD, a series of drug fragments is first screened and then a few active fragments are linked or merged to construct a much more active lead compound. FBDD has the advantage that only a limited number of compounds have to be examined because the variety can be realized by the combination; on the other hand, it has the drawback that the fragmentation can reduce the binding affinity to make it more difficult to detect the binding modes. Recently, we proposed a new computational method for ligand mapping on protein surfaces (3D-RISM-LM) based on the molecular theory of solvation known as 3D-RISM. In the method, ligand is regarded as a solvent and the 3D-spatial distribution functions of ligand atomic sites are used to identify the most probable binding modes (MPBMs) of ligand molecule. In this study, 3D-RISM-LM is applied to FK506-binding protein to demonstrate the usefulness of the method. In the calculation, the MPBMs of several fragments that correspond to functional moieties of the known inhibitors are obtained. On the basis of the results, we discuss the practical ability of FBDD using 3D-RISM-LM. Discussion includes whether the fragment MPBMs can identify the inhibitor-binding sites, whether linking and/or merging of them can reproduce the known inhibitors, particularly the common anchor motif, and whether they can predict the effective direction of fragment growth.

1P044 ラミナリン結合におけるエンド-1,3-β グルカナーゼの糖結合モジュールにあるアスパラギン酸3残基の役割 Role of three Asp residues in carbohydrate-binding module of endo-1,3-β-glucanase in laminarin binding

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We have analyzed the polysaccharide recognition by carbohydrate-binding module (CBM) attached to the C-terminal region of a catalytic domain of endo-1,3-β-glucanase from *Cellulosimicrobium cellulans* DK-1. The CBM, CBM-DK, is classified as CBM family 13, and would consist of a tandem of three imperfect repeats. We overexpressed CBM-DK, corresponding to Gly257-Leu383, in *E. coli* with N-terminal His-tag, and purified it using an affinity chromatography. In the same way, we also prepared its mutants, in which Asp270, Asp311, and Asp355 were substituted to Ala, individually or simultaneously, to analyze the role of Asp residues in the laminarin binding. The circular dichroism and NMR experiments indicated that the purified CBM-DK has β-strand-rich structure. The laminarin binding was analyzed using Biacore, in which the CBM-DK and its mutants were immobilized on the sensor chip. Among the single Asp substituted mutants, the laminarin binding affinity of D270A mutant was significantly reduced. In addition, the double Asp substituted mutant, D311A/D355A, retained the ability of laminarin binding. These results indicate that Asp270 is most critical, and the first repeat of CBM-DK is much more favorable than other two repeats. This is contrast to the assumption that each repeat of CBM equally contributes to the polysaccharide binding. On the presentation, we will discuss the laminarin recognition mode of CBM-DK, together with the results of affinity electrophoresis and isothermal titration calorimetry measurements.

1P045 ナノシリカとナノダイヤモンドの表面における BSA のスレッシュホールド吸着作用の構造と秩序 Conformational Threshold Adsorptions of Systems BSA and Nanosilica/diamond

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From the fluorescences (excitation at 285 nm) of BSA(SIGMA) molecules of 0-1000 as well as 0-10000 nM in 7 mM PPBS at pH = 4.70 before and after adsorption reactions on the surfaces of nanosilica(NS) and nanodiamond(ND) of diameter 100 nm from the suspension solutions(50μg/20μL), the adsorption thresholds, reaction constants as well as coverages have been obtained. Ad-