obtain from the HPLC analysis. The results reveal that the aging-related increase of D-amino acids occurs about PP1C, which is a catalytic subunit of PP1 (Serin/Threonine phosphatase 1). PP1C influences structure and function of target proteins through dephosphorylation. Tau, which is one of the most important molecules causing Alzheimer's disease, is also the target of PP1C. Hyperphosphorylation of Tau is thought to be one of trigger of Alzheimer's disease. Our results suggest that the increase of D-amino acids in PP1C may induce its conformational change, which may contribute to hyperphosphorylation of Tau through the inhibition of PP1C dephosphorylation function and may be the onset of Alzheimer's disease.

3P-008 ヒト由来アрилカーボンリセプタダイオキシン結合ドメインの構造モデリングとその動的構造の解析 Structural Modeling and Dynamical Properties of Human Aryl Hydrocarbon Receptor Dioxin Binding domain

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The aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix Per-Arnt-Sim (bHLH/PAS) family proteins, and is a transcription factor activated by structurally diverse ligands including dioxins, which is known to be nongenotoxic carcinogens. Due to its serious toxicities, it is referred to as an environmental hazard. Despite of the serious significance, experimental structures of AhR have not been determined so far; the binding mode of the dioxin in AhR remains to be elucidated. In this study, we constructed the structural model of the ligand binding domain (PAS B domain) of the human AhR (hAhR) for the first time, using homology modeling techniques coupled to molecular dynamics simulations.

We have identified an intramolecular cavity in the core region of hAhR. Notably, the size of this cavity is significantly larger than those present in the related proteins, whereas the folds are well conserved. Thus, a dioxin molecule is likely to be well fitted to the cavity. Actually, we have preliminary identified candidates of a hAhR-dioxin complex using docking calculations. The MD simulations of the apo hAhR structure have further revealed that the cavity size is regulated by dynamical properties of a loop region, which includes an insertion of a glycine (Gly) residue. The structural flexibility increased by the Gly insertion in AhRs is thus suggested to contribute to the binding mode of the dioxin. The refinement of the complex structure is being performed to evaluate the binding mode by using QM/MM hybrid calculations.

3P-009 蛋白質高次構造会合・解離反応に及ぼす kosmotrope や chaotrope 溶質の影響 Effect of Kosmotropic and Chaotropic Solutes on the Assembly of Protein Macromolecular Structures

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We have studied the assembly of the isolated chains of hemoglobin (Hb) into their tetrameric conformation in the presence of two ionic solutes: phosphate (a kosmotrope, used here as a buffer system) and NaI (a chaotrope that disrupts the structure of water molecules). Structurally, Hb is a homodimer of heterodimers that follow the reaction $2(\alpha)(\beta) \rightleftharpoons (\alpha)_2(\beta)_2$. However, the reactions involved in the formation of the $(\alpha)(\beta)$ dimer are much more complex, since they involve the dissociation of dimeric α chains and tetrameric β chains.

When optical absorption spectra in the visible region of isolated α and β chains were compared with the absorption spectrum of the tetrameric form, we detected two peaks around 540 and 580 nm. This indicated that the tetramer formation was accompanied by an increase in optical absorption, which can be considered a signature for the assembly of the dimeric α and tetrameric β chains into their new quaternary structure. Upon addition of 2 M NaI, we detected a decrease in intensity of both the peaks. Results from other experiments, such as size exclusion chromatography, suggested the fact that iodide ions promoted the dimerization of tetrameric Hb.

To confirm the chaotropic effect of iodide, we followed the changes in optical absorption over time (300 s range) of the mixing of isolated α and β chains in the absence and presence of IHP, and compared them against those obtained under stripped conditions (only phosphate present). We detected no changes for stripped conditions either in the absence or presence of IHP. It is apparent that iodide ions in solution in concentrations as high as 2 M perturb the interactions that stabilize macromolecular assemblies, most likely hydrophobic interactions, without inducing protein denaturation.

3P-010 ナトリウム結合部位を標的としたトロンビン阻害剤の設計に関する研究

A study on the design of thrombin inhibitors targeting the Na⁺ binding site

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Thrombin is a multifunctional trypsin-like protease that plays a central role in the blood coagulation system. Thrombin mediates the conversion of fibrinogen to fibrin, which polymerizes to form a fibrin clot. Thus, thrombin is considered to be a major target protein for the development of anticoagulant.

Thrombin has three sites of action, active site, substrate binding site and Na⁺ binding site. In the conventional strategy, active and substrate binding sites have been targeted for the design of thrombin inhibitors. Thrombin is an allosteric enzyme existing in two forms, slow and fast, targeted toward anticoagulant and procoagulant activities. The slow to fast transition is induced by Na⁺ binding to the site. The aim of this study is to design novel thrombin inhibitors which directly inhibit the Na⁺ binding site.

We have revealed that isopropanol binds to the Na⁺ binding site of thrombin. In the crystal structure of thrombin-isopropanol complex we have determined to 2.8 Å resolution, an isopropanol molecule is found buried in the Na⁺ binding site. This gives us detailed information on the Na⁺ binding site and strategy to design of thrombin inhibitors targeting the Na⁺ binding site.

3P-011 化合物の自動サーチとフィッティング方の開発

A method of locating and fitting ligands for X-ray crystallography

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With the technique progress in X-ray crystallography, the time consumption on obtaining the structure model of a protein or a protein-ligand complex in an atomic scale has become shorter and shorter. This reduction in time consumption made the structure based drug design (SBDD) a feasible method in the study of drug discovery. Compared with the classic methods of drug design, SBDD is advantageous in refining the ligand library on base of the analysis of the known protein structure so that the efficiency of discovering a drug can be dramatically improved. Even though, in the refined library, there are still hundreds or thousands ligands required to be screened for crystallization and structure analysis. This analysis is still a time-consuming process. Therefore, the request for automating this process becomes urgent. Rooted on the software LAFIRE, which was developed for the purpose of automating the process of refining protein structure model, a method aiming to automatically locate and fit ligands to the known protein structures with the reference of the electron-density map has been developed. In this method, the blobs suitable to an inputted ligand are picked out firstly. Then, the ligand will be automatically divided into several inflexible elemental parts and these elemental parts will be located among the blobs in a distributed manner. Finally, the distributed elemental parts will be recombined and evaluated to find a solution of the fitted complete ligand. This method has been incorporated into LAFIRE as a new function module of Ligand Detector (LDetector) and tested in sets of data from our laboratory and protein data bank. For most of them, the correct structures of the protein-ligand complexes have been achieved automatically. In this presentation, we will report some results and make a discussion on them.

3P-012 新規フォールドに対する立体構造予測に向けて ~フラグメントアセンブリ法による予測精度のライブラリ依存性~ Toward the Success of Template-Free Protein Structure Prediction - Dependency of Performance on the Fragment Libraries-

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The prediction of the 3-dimensional structure of a protein from its amino-acid sequence alone, often called De Novo structure prediction, is one of the most important and challenging problems in biophysics today. Although a great deal of effort has been put into this issue, the present status of the ability of De Novo prediction method is far from practical use, as shown in the result of CASP8. In this presentation, we will describe our attempt to improve the accuracy of De Novo protein structure prediction method.

Currently, the most successful method for De Novo prediction is the fragment assembly method, in which tertiary structures of a target protein are generated by assembling fragments extracted from PDB. We have qualitatively shown that the performance of fragment assembly method strongly depends on the conformational space defined by fragment libraries. However, quantitative criterion of successful fragment libraries is still unknown. Here, we try to identify the quantitative criterion by performing fragment assembly structure prediction, using various kind of fragment libraries. Especially, we will focus on the following two quantities of fragment libraries. (1) how similar fragment structures to the native must be included in libraries for successful prediction. (2) how many dissimilar fragments (noise) can be included in libraries for success. Detailed results and discussion will be given in the presentation.

3P-013 文脈依存スコアによる蛋白質構造予測

Context-specific score for protein structure prediction