3P040 モノクローナル抗体を用いたタンパク質の構造変化の解析
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A method for the detection of conformational change at neutral pH region by monoclonal antibodies
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Human serum albumin (HSA) is one of the most abundant proteins in human plasma. HSA has two binding sites and binds with drugs, metals, amino acids, fatty acids, bilirubin and so on in these sites. HSA undergoes the conformational change at pH 6-9 region (N-B transition) and it affects the binding to these ligands. Although the N-B transition is very important for interaction between HSA and ligands, the difference of the N and B conformation has not been revealed. We tried to clarify the difference of two states using monoclonal antibodies.

At first, to determine the epitope of anti HSA monoclonal antibodies, we applied the yeast display system. The messenger RNA was extracted from human liver library (TaKaRa) and whole HSA and HSA subdomains were amplified by specific primers. These fragments were expressed on the yeast surface and the epitope mapping was performed by FACS. Next, the conformational change induced by pH was examined by ELISA using epitope determined monoclonal antibodies. Binding of HY4, HY5 and HY11 (anti-HSA monoclonal antibody) to HSA at pH7.5-9.0 range was weakened by an increase in pH from 5.0 to 9.0. The epitope of these antibodies was defined to be around drug binding site I. The antibodies which recognize other subdomain showed no significant change in the binding to HSA at pH5.0-9.0 range. In conclusion, the N-B transition is occurred around drug binding site 1 and it affects the affinity of HSA to ligands like bilirubin.

3P042 ジヒドロ葉酸還元酵素におけるフォールディングエレメントや活性部位の接続部位に含まれる情報の抽出
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Elucidation of the information encoded in the regions connecting folding elements and/or active site of dihydrofolate reductase
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It is now widely accepted that the entire region of primary sequence is not equally important for the structure formation and function expression. In some proteins, amino acid sequence can be simplified. Iwakura et al. proposed that the essential regions for the structure formation that called folding element are distributed through the entire sequence of dihydrofolate reductase (DHFR). They demonstrated that the mutations in the folding element affect the folding reaction, whereas the mutations the other regions do not. It is obvious that the substrate or cofactor binding regions and the active site bear the information on function. In order to examine what information is encoded in the regions other than folding elements and functional sites of DHFR, we applied a systematic alanine insertion method to these regions. We showed that important regions for the functional expression and/or structure formation for region 1-65 in DHFR can be derived this method. The present study is also a part of the systematic alanine insertion analysis of DHFR. We selected the regions from 66 to 85 and from 133 to 159 which include a few folding elements with relatively short length (3-5 residues). Now we are performing the TMP resistance assay and the solubility assay to characterize the alanine insertion mutants. The role of these regions to structure formation and activity will be discussed.

3P041 網羅的アラニン挿入変異法によるジヒドロ葉酸還元酵素の構造形成および機能発現領域の抽出
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Extraction of the regions encoded foldability and/or functionability from dihydrofolate reductase by a systematic alanine insertion
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Using staphylococcal nuclease (SNase), we demonstrated that the regions encoded foldability and/or functionability, called the structural and the functional elements, respectively, can be extracted by a systematic alanine insertion analysis. Each element is comprised of several continuous residues up to 10 or so on the sequence. We assume that the elements act as fundamental units for structure formation and activity. The folding elements of dihydrofolate reductase (DHFR) were proposed by Iwakura et al. using a systematic circular permutation. In order to compare between the two analyses, and between the folding elements and the structural elements, we have applied the alanine insertion analysis to DHFR. We made every possible alanine insertion mutants from the 85th to the 132nd residue of DHFR. This region contains three folding units (90-104,106-118,123-125). The DHFR activity of each alanine insertion mutant was screened by the TMP resistance assay, and the foldability of each mutant was examined by the solubility assay. Consequently, the alanine insertion for the regions of 92-103, 108-114, 122-123, and 125-126 led to the loss of the activity, and the insertion for the regions of 90-100, 102-113 and 128-129 caused the loss of the foldability. These regions are almost overlapped with the folding elements. From these observations, we conclude that the systematic alanine insertion analysis can be applied in general, and that the extracted elements should be fundamental units for structure formation and activity.

3P043 アミロイドベータペプチドにおけるアミロイド線維形成に対するグリシンの役割
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Role of Glycines on Amyloidogenesis of Alzheimer's Amyloid-Beta Peptide Fragments
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Amyloid β -peptide (A β) is comprised of 39 to 43 amino-acid residues and has tendency to form amyloid fibrils, which are associated with Alzheimer's disease. In these amyloid fibrils, A β is perceived to have an intermolecular β -sheet structure. Furthermore, A β (29-42), which is comprised of the residues 29-42 of A β , also has an intermolecular (antiparallel) β -sheet structure in aqueous solution.

The multicanonical-multioverlap algorithm has advantages of both the multicanonical algorithm and the multioverlap algorithm [1-3]. Namely, the multicanonical-multioverlap simulations can sample effectively the conformational space including specific configurations. Applying this method to protein systems, therefore, we can obtain effectively the accurate free-energy landscapes of these systems including specific configurations. In this poster, we report the results of our application of the multicanonical-multioverlap algorithm to A β (29-42) dimer with implicit water. By employing a β -sheet structure of A β (29-42) as a reference conformation in the multicanonical-multioverlap simulations, we study the secondary structures and aggregation properties of A β (29-42). Furthermore, we plan to discuss the role of glycines on amyloidogenesis of A β (29-42).

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