1P086 データベース解析を利用した、抗体の抗原結合フラグメントにおける高次構造決定因子の探索
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Search for determinants regulating the quaternary structures of Fv fragments of antibodies through a database analysis
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The structures of Fv and Fab fragments have been accumulated by X-ray crystallography. The quaternary structures of Fv fragments, i.e. the association modes of the variable domains of heavy and light chains, are in variety with respect to the orientation of the domain axes and their distances. Comparative inspections have suggested the existence of any structural determinants regulating the association modes of the variable domains (Nakasako unpublished results). Because the association mode of variable domains determines the spatial distribution of six complementary determining regions (CDRL1-L3 and H1-H3), a search for structural determinants is important to design the structures of antibodies for chemical engineering and therapeutics. In the present study, we made a program code to characterize the quaternary structures of Fv portions and to extract common structural features. Through applying the analysis, we found variable domains of heavy chain, in particular CDRH3, have great influences to the association of variable domains. Further analysis to specify which portion of H3 is important is currently under way.

1P088 固体NMRによる膜表面タンパク質 PLC-δ1 の局所運動性の解析
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 1aC01 **Study of Local Mobility of peripheral membrane protein PLC-δ1 by Solid State NMR**
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We examined local mobility of phospholipase C-δ1 (PLC-δ1) pleckstrin homology (PH) domain bound to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) embedded in POPC fully hydrated multi-lamella vesicle (MLV). Motionally averaged ¹³C-¹H dipolar interactions of [3-¹³C]Ala-labeled PLC-δ1 PH domain were studied by an amplified separated local dipolar field solid state NMR spectroscopy under magic angle spinning. Dynamic order parameters (DOPs) thus obtained for PLC-δ1 PH domain as viewed from Ala residues were determined by a normalized dipolar coupling constants with respect to those of static ones. The DOPs for the main peaks of Ala116, and 118 have been obtained as 0.0556 and 0.0553, respectively. Compared to DOP of 0.33 in Ala crystal and transmembrane regions of bacteriorhodopsin, DOPs for Ala116, and 118 residues in PLC-δ1 PH domain exhibited less than 20% of those. And DOPs for other Ala residues were under detection limit with current S/N ratios of spectra, thus those may have further smaller DOPs. Consequently, the results indicate physiologically active sample condition of PLC-δ1 PH domain bound to fully hydrated MLV has extremely higher mobility than transmembrane proteins. The differences of motilities among Ala residues in PLC-δ1 PH domain gives further insights of structural feature. The relationship of local mobility of PH domain and its function will be discussed.

1P087 タンパク質－ペプチド相互作用：ペプチド結合に対するエピトープ様タンパク質表面領域
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Protein-peptide interaction: Epitope-like protein surface areas for peptide binding
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An antibody binds to a specific surface area of an antigen, called 'epitope'. Similarly, a peptide binds to a specific surface area of a protein, accordingly 'epitope-like' one. Therefore, the whole surface of a protein might be covered by an ensemble of such peptides, leading to a kind of decomposition of a protein surface into epitope-like areas. If we think about the finiteness, though a very large number, of the protein surface structure, registering such couples of an epitope-like area of a protein and its cognitive peptide may be useful for predicting possible binding peptides to a particular protein, of which surface structure is known. This idea can be experimentally approached by an exhaustive survey of peptide binding to a protein, which had been performed in our experiment to find protease inhibitory peptides. Tens of seemingly independent peptides were found to bind to the cathepsin E protein. We are investigating whether some of them are truly independent binders to the protein or whether they are recognizing independent epitope-like areas, by way of a binding assay using peptide-linker-peptide-structured molecules. Theoretical and experimental aspects will be viewed on this subject. This study was supported partly by the fund from the Rational Evolutionary Design of Advanced Biomolecules (REDS) Project, Saitama Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence supported by JST.

1P089 多次元固体NMR法による膜蛋白質 *pHtrII* の構造解析
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Structural analysis of transmembrane halobacterial transducer *pHtrII* by multi-dimensional high-resolution solid-state NMR
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Transmembrane protein *pHtrII* is a transducer which binds to phoborhodopsin. The light excitation of phoborhodopsin is transmitted into the cytoplasm through *pHtrII* to promote negative photoaxis. We studied uniformly ¹³C, ¹⁵N labeled 159-residues *pHtrII* by high-resolution solid-state NMR. The *pHtrII* (1-159) was reconstructed into the deuterated DMPC membranes. High-resolution solid-state 2D NMR was measured under magic-angle spinning. NMR spectra were recorded at the ¹H resonances of 500, 600 and 700 MHz. At first, we have assigned ¹³C signals of *pHtrII* (1-159) by using ¹³C-¹³C spin diffusion under DARR at the mixing time of 5, 10, and 200 ms. Intra-residue correlations across 1-2 bonds were observed at short mixing time. At a long mixing time of 200 ms, intra-residue C^α correlations across 3-bonds were obtained. Most amino acids except Tyr and Pro were assigned. These result suggested that *pHtrII* (1-159) mainly forms α-helix structure. All ¹³C signals could not be observed in these dipolar-coupling-based cross-polarization methods because of large-amplitude molecular motions at room temperature. We performed *J*-coupling-based HC-INEPT and HCC-TOBSY experiments in order to obtain signals of mobile protein segments. Many signals in mobile regions were observed in HC and HCC 2D spectra.