9:00~12:00 D会場 / Room D

蛋白質_構造機能相関 2

Protein: Structure & Function 2

3D0912 An extensively hydrated and folded conformer stabilized at high pressure and low temperature. The case of c-Myb R2 sub-domain.

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In order to find out the intrinsic conformational flexibility of the c-Myb R2 sub-domain, here we study its conformation using variable-pressure Trp fluorescence and ¹H NMR at low temperature (5°C). We used a chemically stable pseudo-wild type C130I mutant (WT) retaining its internal cavity and a cavity-filled mutant (C130I/V103L).

The combined fluorescence of the three Trp residues in the core of WT showed a red shift of $\lambda_{\max}$ from 342 to ~355 nm as pressure was varied from 3 to 300 MPa, and $\lambda_{\max}$ remained the same up to 600 MPa. The result indicates that all the Trp side-chains are in a fully polar environment above 300 MPa, which is normally taken as indicating that the protein underwent full unfolding. On the other hand, the high-pressure ¹H NMR spectra of WT showed continuous shifts of the high-field shifted methyl signals characteristic of the hydrophobic core toward still higher field, which clearly indicates that the protein remained folded with a compaction of the core even at 300 MPa. These two experiments suggested the existence of a peculiar conformer (N') at 300 MPa that is extensively hydrated, but, on average, folded. The analysis of ¹H NMR chemical shift changes with pressure further suggested that the conformer N' is in equilibrium with the known conformer N stable at 0.1 MPa. N' was not found in the cavity-filled mutant, suggesting that it is intimately related to the cavity hydration. We speculate that N' is likely to be the functional conformer that facilitates the DNA binding of R2.

3D0924 隣接炭素化学シフトへの重水素同位体効果を利用したタンパク質側鎖 OH/SH 基の新しい NMR 研究手法
New NMR method for studying the side-chain OH/SH groups in proteins using the deuterium isotope effect on adjacent carbon chemical shifts

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Hydrogen bonds involving side-chain functional groups in proteins are closely related to their structural stabilities and biological functions. Presumably, the slowly exchanging hydroxyl (OH) or sulfhydryl (SH) groups of Ser, Thr, Tyr and Cys residues are involved in hydrogen bonds, and thus play structurally important roles. We have recently developed a robust new NMR method for detecting slowly exchanging OH/SH groups and measuring their hydrogen exchange rates. In our approach, the Ser, Thr, Tyr and Cys residues, which are synthesized with optimized isotope-labeling patterns, are selectively incorporated into a protein, by either conventional E. coli synthesis or a cell-free expression system. By virtue of the designated isotope-labeling patterns of these amino acids, we could unambiguously assign and identify the residues bearing slowly exchanging OH/SH groups, simply by searching for discrete isotope shifted ¹³C-NMR signals for the carbons directly bonded to the OH/SH groups, in a protein dissolved in an equimolar H₂O/D₂O mixture. The results for the 18.2 kDa E. coli peptidyl prolyl cis-trans isomerase (EPPIb) revealed that about 30 % of the OH/SH protons in this globular protein were slowly exchanging. We successfully elucidated their hydrogen bonding patterns, based on NOEs involving their OH/SH protons, and determined the precise side-chain conformations. In this presentation, we also describe the application of this method to the analysis of a protein-ligand interaction involving side-chain hydrogen bonds.

3D0936 Prediction of dynamical conformation of biantennary complex-type N-glycan in water by replica-exchange molecular dynamics simulations

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Oligosaccharide chain plays important roles in the development and function of most living organisms. The activity in the above processes may depend on the dynamical conformation of glycans. However, the precise structure of oligosaccharide is hardly tracked by the conventional structural analysis, such as X-ray crystallography or NMR spectroscopy. The inherent flexibility of the glycan molecule even makes conventional molecular dynamics simulations difficult to sample the possible conformational space sufficiently. Here we perform replica-exchange molecular dynamics simulations with explicit water solvent to reveal the dynamical conformation of a typical biantennary complex-type N-glycan. We successfully address the multi-modal equilibrium conformation of the N-glycan with several distinct minima (backfold, intermediate, and extend forms). We also clearly show the conformational shift upon the introduction of bisecting GlcNAc. We will discuss all details including H-bond analysis as well as its biological implications.

3D0948 CS-PCA 法 (化学シフト主成分解析) を用いた蛋白質-多分子基質結合系の解析

Application of CS-PCA (Chemical shift-principle component analysis) to the NMR data on a multiple-ligand-binding system

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The chemical shift changes in NMR spectra provide useful information about the interaction of a protein with its ligands. However, in a multiple binding system, determining binding sites, stoichiometry and binding constants (K_b) is difficult. Here, we introduce a method named CS-PCA, in which a principal component analysis (PCA) is applied to the chemical shift (CS) data of the NMR titration experiment. CS-PCA can draw concerted chemical shift changes for several residues closely positioned in the 3D structure, illustrating the locations of distinct binding sites. We applied CS-PCA to the NMR spectral data obtained from the titration of bovine β -lactoglobulin (β LG) with 1-anilinonaphthalene-8-sulfonate (ANS) as a model of the multiple ligand system. Upon the titration with ANS, some peaks of HSQC shifted with curvilinear trajectories, indicating the existence of more than one binding step. Based on the result of CS-PCA, we conclude that β LG has two binding sites (sites 1 and 2) and determined their K_b values. It was found that two ANS molecules bind at site 1 cooperatively with a partial unfolding of the β LG structure, whereas the binding of an ANS molecule to site 2 occurs without a conformational change. From the present results, this method was confirmed to provide not only the positions of binding sites and the K_b values but also information about the binding mechanism. Thus, it is anticipated to be a general method to investigate the protein-ligand interactions.

3D1012 粗視化力場 MARTINI を用いたタンパク質-リガンド結合シミュレーション

Protein-ligand binding simulation with a coarse-grained force field, MARTINI

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Clarifying the mechanism of protein-ligand interactions is one of the most important research subjects in the field of biophysics. However, most of the research efforts have been devoted to developing new algorithms and programs to predict the docking structure. The process of the ligand binding remains to be clarified. Molecular dynamics simulation is a straightforward way to study the ligand-binding process. However, reproducing the ligand-binding process in an all-atom simulation is quite difficult because it requires a very long time simulation. For this reason, we explore the possibility of coarse-grained simulation.

In this study, we used the MARTINI force field, in which four non-hydrogen atoms are mapped to one particle on average. We performed simulations for several protein-ligand pairs for which the complex structures are known. Each simulation system was composed of one protein molecule, randomly placed ligands, and explicit water solvents. The simulations were repeated several time