Previously, we studied correlation between the melting of 2D-crystalline array of bR and the photobleaching. The results suggested that the weakening of intermolecular interaction is responsible for irreversible photobleaching. In this work, we carried out the measurement of visible circular dichroism (CD) spectra and the photobleaching experiment of bR reconstituted into three kinds of phosphatidylcholine (PC) vesicles with different acyl-chain length. Visible CD spectra showed an exciton-to-positive transition of bR reconstituted into dimyristoyl-, dipalmitoyl- and distearoyl- PC vesicles around 17, 35 and 50 °C, respectively. These transition temperatures were close to the main transition of the reconstituted vesicles measured by calorimetric measurement. Irreversible photobleaching was started to occur at the same temperature regions as the change in the visible CD transition, clarifying the correlation between the melting of crystalline assembly and the photobleaching of bR. On the other hand, the thermal bleaching in the dark was not observed below 60 °C for each sample. The plausible model for irreversible photobleaching is discussed, based on these experimental results.

3P120 NADH-ユビキノン酸化還元酵素の FMN と鉄-硫黄クラスターの酸化還元状態による共鳴ラマンスペクトルの変化 Resonance Raman spectrum changes of FMN and iron-sulfur clusters of NADH-ubiquinone oxidoreductase upon redox states

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NADH-ubiquinone oxidoreductase (complex I) located at the entrance of mitochondrial electron transfer chain is the largest component of the respiratory chain with the proposed mass of 1000 kDa. The enzyme transfers electrons from NADH to ubiquinone with 10 isoprene unit (Q10) coupled with proton-pumping. The redox-active sites include one FMN, eight iron-sulfur clusters and one tightly bound ubiquinone. In order to elucidate the reaction mechanism of complex I, spectral characterizations of these redox sites are prerequisite as well as structural analyses at high resolution. Resonance Raman spectroscopy has provided a practical method for observing selectively the vibrational spectra of chromophoric groups of biomolecules. The resonance Raman spectra of the FMN and iron-sulfur clusters of complex I upon excitation at 441.6 nm and 488 nm have been determined by using our highly improved enzyme preparation and resonance Raman apparatus. In order to detect the changes of the spectra upon the change of redox states, the NADH-reduced complex I was prepared. For preventing the reoxidation of reduced enzyme, a spinning cell was filled with Ar gas and sealed with silicon cover. NADH concentration have to be maintained constant during the spectrum accumulation for 180 min. The addition of malic acid, malic dehydrogenase, citrate synthase and Acetyl CoA to NADH-reduced complex I was effective for maintaining the NADH concentration constant to keep complex I reduced.

3P121 Conformational change of mouse ABC transporter ABCB1 from an inward-facing conformation as studied by molecular dynamics simulation

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ATP-binding cassette (ABC) transporters are a large family of membrane proteins which transport various substrates by using MgATP hydrolysis. ABCB1 plays an important role in acquiring multidrug resistance. The x-ray structure of ABCB1 (PDBID 3G5U) was reported in 2009 for the first time, which enables us to study how it works based on the atomic-level structure. In this study, we use molecular dynamics simulation to follow conformational changes of ABCB1 starting from an inward-facing conformation and to reveal what intermediates it goes through in the transition from the inward-facing conformation to an outward-facing conformation. In addition, we also discuss the effect of an inhibitor on such structural changes. After 20 ns MD simulation, it was found that nucleotide-binding domains (NBD) underwent large conformational changes. Consequently, two NBDs were dimerized in a unique fashion that only one of two ATP binding pockets was close. In order to analyze what mode of molecular motion induces such conformational changes, we carried out principal component analysis for the trajectory data. As a result, it was found in the first principal component that one of the two NBDs rotate in clock-wise fashion and the other in anticlock-wise one with respect to the membrane normal, leading to the asymmetric contact between the two NBDs as described above. These results were obtained for the ATP-unbound state of ABCB1 transporter. We are under investigation now for the ATP-bound state.

3P122 全原子分子動力学シミュレーションによる多剤排出トランスポーター AcrB の解析 All-atom molecular dynamics simulation of multidrug efflux transporter AcrB

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In E. coli, it is known that the tripartite multidrug efflux system (AcrB/ AcrA/ TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each subunit is different conformation, "binding state", "extrusion state" and "access state". Especially, only extrusion state subunit has different side chain conformation of residues (Asp407, Asp408 and Lys940), which are essential for proton translocation (protonation site). These results suggest that drugs are exported by a three-step structural change involved in proton motive force which is inferred to be caused by change of protonation states of the protonation site residues. However, the structural change process which involved in proton motive force is still unclear. In the present study, we performed 100 ns all-atom molecular dynamics (MD) simulations of three types of AcrB-membrane-water systems which are different from protonation state of Asp407 and/or Asp408 in extrusion state protomer. During all of the 100 ns MD simulations, the global structures of each subunit were conserved. However, in extrusion state protomer, the conformation of the protonation site residues were changed to those of other state protomers only when Asp 407 and 408 residues were deprotonated. In this presentation, we will discuss the structural change and proton transfer mechanism.

3P123 膜貫通タンパク質ドメインアーキテクチャー解析のための新規ドメインファミリー定義 Defining Novel Domain Families for Comprehensive Analysis of Domain Architectures in Transmembrane Proteins

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Transmembrane (TM) proteins have the characteristic domain architectures, consisting of a TM domain and a few soluble domains carried on long loops with more than 60 residues in most cases. Having the domain architectures determined for a large number of TM protein sequences, we would be able to understand functional makeup of TM proteins and evolutionary pathways to extant TM proteins. We first, in this study, assigned the Pfam and SCOP domains to loop regions of 62,348 TM protein sequences from 25 genomes, and then we defined novel domain families on the remaining long loop regions without assigned Pfam nor SCOP domains.

A total of 29,807 sequences (47.8%) have at least one Pfam or SCOP domain assigned, and the remaining 32,541 sequences have no assigned domains. After removing the domain-assigned segmental loop regions, we had still 66,258 loop segments remaining, which are longer than 60 residues with being able to carry soluble domains. Applying the MKDOM algorithm to these remaining loop segments, we defined 17,015 novel domain families (with ≥ 2 family size), with the result that multi-domain TM proteins to be the targets of the domain architecture analysis increased 10,487 (16.8%, Pfam/SCOP) to 14,249 (22.9%, considering the novel domain families).

We are now trying to analyze comprehensively the domain architectures in TM proteins, and also to investigate the evolutionary pathways of TM topologies through analyzing domain family sharing among TM proteins with the different number of TM segments.

3P124 全原子および粗視化MD計算による F_o のプロトン経路と c-ring 回転運動の解析 Proton pathway and rotary motion of c-ring in F_o analyzed by all-atom and coarse-grained molecular dynamics simulation.

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F_o is a membrane-embedded rotary motor, functioning as a part of ATP synthase, and is composed of *a*, *b* and *c* subunits. Transmembrane electrochemical potential of proton (or sodium ion) drives the rotational motion of *c*-ring relative to the *a*-subunit. The torque-generation mechanism, however, remains speculative and even the proton translocation pathway is not clear. To shed light on these issues by a computational approach, we conducted molecular dynamics (MD) simulations employing all-atom and coarse-grained models. In the all-atom model, *a*-subunit (PDB ID:1C17) and five *c*-subunits (PDB ID:2WIE) were embedded into DOPC bilayer sandwiched by water layers

containing ~150 mM NaCl. In the coarse-grained model, the equilibrated structure resulting from the all-atom MD was used as the input structure. To consider higher energetic penalty of a charge in a lower dielectric environment, we introduced a coarse-grained Born-energy model that enables a charged particle (representing an oxonium ion or a sodium ion in this study) to sense its surrounding dielectric environment in a coarse-grained manner. We first analyze the all-atom MD data focusing on the stability of the α -subunit, relative rotational motion of the c -ring, emergent aqueous channel(s) and the distribution of sodium ions. We then analyze the coarse-grained MD data primarily focusing on the pathway of charged particles, making comparisons with the putative proton channels.

3P125 Ionic effect on MD-SAXS profile

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The combination of small-angle X-ray solution scattering (SAXS) experiment and molecular dynamics (MD) simulation is now becoming a powerful tool for studying protein structures in solution at an atomic resolution. Several studies have developed the calculation methods of SAXS profile from protein atomic structures, in which scattering from hydration structure around the protein is considered implicitly or explicitly. Although general SAXS experiments of protein solutions are carried out at certain ionic concentrations, in these calculation methods, ionic effect on SAXS profile has not been considered explicitly. In this study, we investigate ionic effect on the SAXS profile by using the MD simulations of hen egg white lysozyme at various NaCl concentrations.

At 0 mM NaCl, the calculation of the SAXS profile converged completely within ~200 ps MD simulation, but at concentrations larger than 100 mM NaCl, ~10 ns simulation was necessary to obtain the convergence due to large density fluctuations in the bulk region. We also observed certain dependencies of SAXS profile on NaCl concentrations. These results indicate that MD simulation at large NaCl concentrations is a disadvantage in obtaining accurate SAXS profile. To accommodate this problem, we investigated the effect of ionic concentration on solvation structure around the protein, and based on the obtained information, have developed the new calculation method that incorporates the effect of ionic concentration in the calculation of SAXS profile from the simulation at 0 mM NaCl.

3P126 減衰全反射赤外分光法による生体分子水溶液中の水の水素結合状態 Hydrogen bonding natures of water molecules in aqueous solutions of biomolecules by Attenuated Total Reflection Infrared spectroscopy.

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In order to study natures of water molecules interacting with biomolecules in aqueous solutions, Attenuated Total Reflection Infrared (ATR-IR) spectroscopy was conducted on aqueous solutions of protein (bovine serum albumin: BSA), DNA, glucose and glycerol. IR absorption bands from 3000 cm^{-1} to 3600 cm^{-1} due to OH stretching vibration could be decomposed into two components around 3200 cm^{-1} and 3400 cm^{-1} . The 3200 cm^{-1} component corresponds to bound water (water molecules with shorter hydrogen bond) and the 3400 cm^{-1} component to free water (water molecules with longer hydrogen bond). In the case of glycerol solutions containing 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100wt% glycerol, their ATR-IR spectra against air background were subtracted by corresponding ratios of glycerol 100wt% spectrum to obtain spectra for water molecules. The OH band of these difference spectra for water could be well fitted by three components around 3200, 3400 and 3600 cm^{-1} . ATR-IR spectra for the other solutions were also treated in a similar way. Changes in OH bands in difference spectra of BSA, DNA, glucose and glycerol with increasing concentrations will be discussed in terms of "free" against "bound" natures of hydrogen bonds of water molecules.

3P127 Dielectric Relaxation Properties of Aqueous Solution of Urea and Its Derivatives

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Urea is a well known denaturant of proteins and affects many biological functions of biomolecules. To understand the molecular mechanism it is of prerequisite to know the hydration property of urea.

The purpose of this study is to measure the hydration properties of urea and its derivatives to know the roles of carbonyl and amine groups. Hence, the dielectric relaxation spectroscopy measurements were performed at 20°C in the frequency range between 0.2 GHz - 26 GHz for 0.1 M - 0.5 M urea solutions and 0.1 M - 0.3 M urea derivatives such as dimethylurea, diethylurea and

thiourea. Only two relaxation components around f_c of 7.5 GHz and 5.3 GHz were observed for urea and thiourea, whereas three relaxation components with f_c of 1.7 GHz, 6.8 GHz and 15 GHz were detected for dimethylurea, and 1.4 GHz, 7.0 GHz and 15 GHz for diethylurea. Since the rotational relaxation frequency is inversely proportional to the molecular dimension the 1.7 GHz (or 1.4 GHz) component can be assigned to the molecular rotation of dimethylurea or diethylurea. For urea the rotational relaxation frequency seems to overlap the relaxation of hydration water at 7.5 GHz. For three samples such as urea, dimethylurea and diethylurea the component around 7 GHz is probably due to the hydration water. The component of 15 GHz for dimethylurea and diethylurea is possibly due to water relaxation slightly slower than bulk water. This study would become a base to understand the molecular mechanism of urea activities.

3P128 ATPの加水分解反応に関わる熱力学量の再検討 -ATP 多荷イオンのプロトン化反応と希釈のカロリメトリー- Reexamination of thermodynamic parameters relating to ATP hydrolysis -Calorimetry of protonation and dilution of ATP multivalent ions-

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For detailed molecular understanding of ATP hydrolysis energy, we should know the physical origins of the thermodynamic parameters (ΔH and ΔS) for protonations and metal-ion associations of reactant/product species that are in single- or multi-valent anionic states. Most of relevant parameters have been tabulated by Alberty & Goldberg based on their extensive survey of literature values and mathematical treatments (1992). There exist a number of uncertainties in their data base, however, because some of the original values of ΔH quoted there were those obtained indirectly by using the van't Hoff equation and others were those measured but under different solvent conditions. We have recently begun to reevaluate the thermodynamic parameters for reactions involving ATP multivalent ions to build a solid foundation for molecular energetics of ATP hydrolysis in collaboration with theoreticians of statistical mechanics and molecular dynamics. In the present work, we measured protonation heats of multivalent ATP anions by ITC at 25 °C, and obtained a positive value for $\text{ATP}^{4-} + \text{H}^+ \rightarrow \text{ATP}^{3-}$ (4.38 kJ/mol) and a negative value for $\text{ATP}^{3-} + \text{H}^+ \rightarrow \text{ATP}^{2-}$ (-16.18 kJ/mol). These values are in accord with those in the table given by Alberty & Goldberg after correction for the effect of ionic strength. Measurement of dilution heats of ATP^{4-} , ATP^{3-} and ATP^{2-} are also in progress. These results will be compared with the hydration properties of those species in water measured by dielectric relaxation spectroscopy and NMR.

3P129 蛋白質逆ミセルのフェムト秒からナノ秒の構造ダイナミクス Structural dynamics in a protein-containing reverse micelle in the time region from femtoseconds to nanoseconds

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The so-called biological water that hydrates a protein is believed to play an important role in protein's function. Protein-containing reverse micelles are a suitable system to explore the effect of the surrounding waters of protein in detail, because the condition of the surrounding waters changes with w_0 ($=[\text{water}]/[\text{surfactant}]$). The reverse micelle contains one protein molecule. On the other hand, proteins work in a cell, which is crowded with many kinds of biomolecules, and so the surrounding of the protein is different from that in an aqueous solution. As a result, the dynamics and reaction of proteins in a cell will be different from those in a dilute aqueous solution. In the present study, bovine serum albumin was dissolved in AOT/isooctane reverse micelle solution. Further, a coumarin dye was used as a fluorescent probe for the structural dynamics in the reverse micelle, where the dye molecule was found to be localized around the surfactant. The time-dependent red-shift of the fluorescence spectra of the dye molecule is ascribed to the solvation process of the water and AOT molecules around the dye, and so it provides information on the structural dynamics in the reverse micelle. It has been shown that the solvation process consists of several relaxation modes in the time range from 100 fs to 10 ns, and that the time constant and the magnitude of each relaxation mode depend on the micelle size, i.e., the water molecule number (about 100 to 10000 molecules), and are affected by the presence of the protein.

3P130 誘電緩和分光法によるDNA・RNAオリゴマーの水和状態解析 Hydration analysis of DNA/RNA oligomers by dielectric relaxation spectroscopy

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