2B1436 ナノ拘束空間中水の揺らぎの温度変化**Temperature dependence of fluctuation of water in a nanometer-confined space**

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A reverse micelle is a nanometer-scale cage filled with water. The confinement effect on the structure and dynamics of water in reverse micelles has been studied by various experimental methods. These studies have revealed that the structure and dynamics of water in reverse micelles are quite different from those in bulk water, and depend on the micelle size. It is well known that the size of the reverse micelle changes with the water-surfactant molar ratio, w_0 ($= [\text{water}]/[\text{surfactant}]$). It is considered to be important to clarify the nature of water in reverse micelles not only for investigation on biological water in a cell but also from the point of view of statistical physics, that is, nanometer confinement effect on a condensed system. One important question is how water confined in such a space behaves with decreasing temperature far below the melting point of water. However, the temperature-dependent behavior of reverse micelles has hardly been studied. Thus, in the present study, we have investigated it by dissolving the dye probe in the nanometer-sized droplet and by measuring temperature dependence of the visible absorption spectrum of the probe molecule. Reverse micellar solution was prepared by use of AOT and isooctane as a surfactant and oil solvent, respectively. The temperature was varied from 293 K to 173K, which is near the melting point of isooctane. The diameter of the droplet was changed from ~ 1 nm to 8 nm by adjusting the w_0 value. The experimental results are discussed in detail in the presentation.

2B1448 (ジメチルスルホキシド・水) 中に見出された、分極が低いにより束縛されている奇妙な水分子**Less Polar but More Bound Water Molecules Found in Binary Aqueous Mixtures of Dimethyl Sulfoxide**

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We have found strange water molecules in mixtures with dimethyl sulfoxide (DMSO).

We have carried out IR measurement of both the O-H stretching and the H-O-H bending vibration modes of the water molecules in the mixtures at various concentrations. We observed $\nu(\text{O-D})$ stretching mode of probe molecules HDO formed in ($\text{H}_2\text{O}/\text{D}_2\text{O}=97/3$ in volume) and $\nu(\text{H-O-H})$ bending mode of the H_2O in ($\text{H}_2\text{O}/\text{D}_2\text{O}=2/3$ in volume).

On increasing DMSO concentrations of the mixtures, continuous blueshifts of $\nu(\text{O-D})$ was obtained, showing that less polar and weaker H-bond donating water molecules were formed. Simultaneously, continuous blueshifts of $\nu(\text{H-O-H})$ was observed over the whole concentration range, showing that bending motions of the water molecules become more restricted against the depolarization of the O-H bonds of the water molecules.

In order to interpret the conflicting results, we carried out IR measurements of symmetric and asymmetric C-H stretching modes of the methyl groups, and observed continuous blueshifts of the modes on increasing water concentration. This shows that the fraction of the C-H groups incorporated in blue-shifting H-bonds, C-H...OH₂, increases with increasing water concentration. The above blueshifts of $\nu(\text{H-O-H})$ can be interpreted inversely as that the fraction of the water molecules incorporated in blue-shifting H-bonds, C-H...OH₂, increases with increasing DMSO concentration. The depolarization of the water molecules is assigned as breakdown of the tetrahedral intermolecular H-bonds network in liquid water.

2B1512 誘電緩和分光法による水和測定の分解能の向上と信頼性の検証**Resolution improvement of hydration measurement by microwave dielectric relaxation spectroscopy and its reliability**

George Mogami, Tetsuichi Wazawa, Makoto Suzuki (*Grad. Sch. Eng., Univ. Tohoku*)

We developed a measurement method of the high resolution microwave dielectric relaxation spectroscopy (DRS), and applied it to hydration analysis of actin, myosin, amino acids, inorganic ions, etc. Recently we reported that ATP and Pi had water whose dielectric relaxation (DR) frequency f_c was 19 GHz close to that of bulk water (17 GHz) (G. Mogami et al., *Biophys. Chem.* 154 2011 1-7.). From the view point of the common DRS resolutions, it would not seem to be a clear difference. We examined the resolution of the present method of Debye decomposition near 17GHz. The model spectra were generated by

mixing dielectric spherical solutes having a Debye relaxation property into water using the Wagner-dielectric mixture theory, and added with random noise having normal distribution with the same standard deviation (SD) as that of the measured spectra. These spectra were analyzed with the present method. The estimation error in f_c was plotted against the amplitude of SD. As a result, when f_c of the model Debye component was closer to 17 GHz, the resolution got worse. As for $f_c = 19$ GHz we verified the resolution of the present system to be less than 0.1 GHz in the DR frequency when the measured SD was a typical value ~ 0.057 .

2B1524 尿素とその誘導体の水のダイナミクスに対する効果のマイクロ波誘電緩和分光法による研究**Effects of Urea and Its Derivatives on the Water Dynamics as Studied by Microwave Dielectric Spectroscopy**

Altansukh Batnasan, Tetsuichi Wazawa, George Mogami, Nobuyuki Morimoto, Makoto Suzuki (*Dept of Mat Proc, Tohoku Univ*)

Urea is known to affect the surrounding water structure in urea-water mixture and to be a denaturant for protein and DNA. Hence, the behavior of an aqueous urea solution has long been studied by various methods (1, 2).

The main aim of this study is to analyze the effect of urea and its derivatives on the dielectric relaxation property of surrounding water. The complex dielectric permittivity of aqueous solutions of urea (0.1-0.5M), thiourea (0.04-0.2M) and alkyl urea derivatives (0.1-0.3M) was measured in the frequency range of 0.2-26 GHz at $20 \pm 0.01^\circ\text{C}$ by high precision dielectric relaxation (DR) spectroscopy. DR components were revealed for urea and thiourea with f_{c1} , f_{c2} and f_{c3} of 18.5-20.3 GHz, 7.3-10 GHz and 2.8-3.7 GHz, respectively, whereas for alkyl urea derivatives with f_{c2} and f_{c3} of 17 GHz and 0.9-1.8 GHz, respectively. Our results indicate that the DR behavior of the solutions of urea and thiourea can be described by the combination of the bulk water and two Debye relaxation functions, which are assigned to be the constrained water and hyper-mobile water (HMW) components. The solutions of the alkyl urea derivatives can be represented by the bulk water and a single Debye relaxation component. The surrounding water molecules around urea and thiourea is more mobile than bulk water, but the mobile water (HMW) is eliminated by alkyl groups in the alkyl urea derivatives.

(1) Idriss, Gerard, Damay, J. Phys. Chem. B. 2010. 114. 4731.

(2) Hayashi, Katsumoto, Omori, Kishii, Yasuda, J. Phys. Chem. B. 2007. 111. 1076.

2B1536 ADP とミオシン S1 の結合熱に及ぼす尿素とその誘導体の影響**P40 Effect of Urea and Its Derivatives on the heat of ADP binding to myosin S1**

Tomohiko Abe, Altansukh Batnasan, Shin-ichiro Yasui, George Mogami, Makoto Suzuki (*Grad. Sch. Eng, Tohoku Univ*)

Urea is known as a denaturant of protein and DNA. Protein denaturation occurs at as high as ~ 5 M of urea. However, the effect of urea on the interaction between a native protein and a ligand is still open to investigation, notwithstanding De Meis (1989) and Wolfenden (1985) have extensively studied ATP hydrolysis in the presence of cosolvents. To understand the energetics of ATP hydrolysis, it is necessary to know the role of water in this reaction. Hydration free energies of ATP and related agents must be indispensable to the free energy of ATP hydrolysis as reported by P. George (1970). If water is involved in the ATP hydrolysis reaction, cosolvents should affect the reaction free energy and enthalpy. In this study, myosin was taken from chicken pectoral muscle. Myosin S1 was obtained by papain treatment. Then we measured the binding heat of ADP and myosin S1 in the presence of low concentration of urea and its derivatives by isothermal titration calorimetry (ITC). The urea concentration range was chosen in accordance with the DRS hydration study by B. Altansukh et al. ADP was injected to 3 mg/mL myosin S1 solution, and the reaction heat was measured at 20°C . In the presence of 0.1 M urea, the binding enthalpy change of ADP was 6.9% larger than that in its absence. We are conducting the measurements of the binding heat in the presence of urea derivatives, and the role of water in the binding of S1 and ADP is to be revealed.

2B1548 脂肪酸アミン／オクタノール溶媒系を用いた ATP, ADP および P_iの水和エネルギー評価**Evaluation of hydration energies of ATP, ADP and P_i using a fatty acid amine/octanol solvent system**