stagnalis. After the CNS was isolated from the body, the cerebral giant cell (CGC), which is one of the regulatory neurons involved in feeding responses, was exposed under a microscope. Then intracellular recording of the CGC was carried out using a glass electrode. The d.c. current injected into the neuron through the electrode was the parameter which controls the neural activity. As the results, in some cases, the neuron revealed the transition from the beating state to the regular bursting state. When the depolarizing current was increased, the increment of the firing rate and the transition to the period-two state, the period-three state and so on were observed. In other cases, however, the neuron revealed the transition from the beating state to the irregular bursting state without the transition to the regular bursting state. In these cases, the complexity became more increased as increasing the depolarizing current. Thus the bifurcation phenomena were different each other among the individuals. Then we reconstructed the attractors from the firing patterns and obtained one-dimensional Poincaré maps from sequential series of intersections. This analysis showed some characteristics of the bifurcation phenomena of firing patterns of the CGC.

3P-244 培養心室筋細胞の引き込み応答における刺激波形の効果

Effect of waveform on the entrainment of cultured ventricular muscle cells

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Entrainment by external forces is one of the most important properties of nonlinear oscillations including our heartbeat. Rhythmic contraction of cardiac muscle cells has been extensively studied from this aspect. In former studies, pulse-wise stimuli have been widely utilized, although it remains unclear whether another waveform of stimulus is more effective for entrainment. Here, we report the effect of waveform on entrainment of rhythmic contractions of cultured cardiac cells. First, we examined the waveform that can entrain the rhythm of a limit cycle oscillator with least power by means of phase reduction. In this way, it was proved that the most effective waveform of the limit cycle oscillator with high orbit stability is identical to that of the phase response curve (PRC). Second, we experimentally studied the entrainment of cultured rat ventricular muscle cells. We used several pulse-wise waveforms and a PRC-like waveform generated from a mathematical model of ventricular muscle cells. As a result, we found that electrical stimuli can entrain the rhythmic contraction of ventricular muscle cells. Surprisingly, we found that a narrow pulse-wise waveform was superior in entrainment to PRC-like waveform and other pulse-wise waveforms. Thus, we expect that PRC of cultured ventricular muscle cells corresponds closely with pulse-wise waveform with a narrow pulse width. Hence, we try to measure the actual PRC of the cultured ventricular muscle cells and examine the properties of cardiac muscle cells from nonlinear dynamics aspect.

3P-245 包埋切片の原子間力顕微鏡観察によるデンプン粒子のナノスケール構造解析

Nanometer-scale structure analyses of food-material by atomic force microscopy combined with resin embedded section.

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Recently, importance of nanotechnology has been increasing not only in science and engineering fields but also in food materials. Actually, several nanometer-sized food materials have been already sold on the market. However, only little information is available for the nanometer-scale structures in food material. Thus, we started the structural analysis of food materials by using atomic force microscopy (AFM) combined with resin-embedded section method. This strategy has advantages that not only enabling the visualization of internal structures and also increasing resolution in AFM observation because the section slice has very flat surface.

We adopted the native starch granules and the micro- to nano-scale processed starch granules as samples. As the results, we successfully obtained topographic images of growth rings with a intervals of a few hundred nanometer, and also detected a few ten nanometer particles. Also by phase image of sonicated starch surface, existence of very fine region with 5 nm width at minimum was detected. Since the detailed structures in starch particles unclarified parts has been still remains, these results would contribute to the starch science and also for nanoscale processing of the starch. Besides these, we also report the development of AFM-aided genome investigation method briefly.

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3P-246 原子間力顕微鏡を用いた酸化ストレスによる細胞肥大過程の力学特性評価

Mechanics of cardiac cells under hypertrophy induced by oxidative stress measured with an atomic force microscope

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Cells change their morphology during migration, different phases of cell cycle and in response to extracellular stimuli. It is known that cardiac cells hypertrophy as some extracellular stimuli such as oxidative stress are applied to them [1]. The hypertrophy is considered to involve alteration in the cytoskeleton [1], however, it is little known how the hypertrophy processes as well as how the physical properties of cells changed under hypertrophy stimulated by the external stimuli. Here we measured the elasticity of cardiac H9c2 cells, after dosing H_2O_2 , with an atomic force microscope (AFM), in which a colloidal probe was used. We characterized the Young's modulus, E , of a large amount of cells cultured on a microarray substrate [2] and culture dish at different concentrations of H_2O_2 as a function of incubation time within 3 days. The results showed that just after dosing H_2O_2 the averaged E significantly decreased with increasing the concentration of H_2O_2 and that the histograms of E exhibited a log-normal distribution. Moreover we found that before the hypertrophy was induced the E began to gradually increase during incubation. We will report detailed relationship among the degree of hypertrophy, the elastic properties and cytoskeletal structures. This work is partially supported by grant program from Enhancing Systematic Education in Graduate Schools, NEDO (06A26007) and the GCOE Program from MEXT of Japan. [1] Q. M. Chen et al. (1998) *Arch Biochem Biophys.* 373 242-248. [2] Y. Mizutani et al. (2008) *Jpn. J. Appl. Phys.* 47 6177-6180.

3P-247 AFM 粘弾性測定によるデキストラン伸長時における中間体の検出

Evidence for the existence of an intermediate state upon stretching dextran captured by AFM viscoelasticity measurement

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Viscoelasticity provides information about the dynamics of molecular motion and/or conformational changes of biopolymers such as nucleic acid, protein and polysaccharide. To investigate the viscoelasticity at single-molecule level, we have developed an atomic force microscopy (AFM)-based method. In this method, a single molecule attached between the cantilever and the substrate is stretched at a slow pulling speed, during which the magnetically-driven oscillatory force was applied to the cantilever and its amplitude and phase information is monitored. The stiffness and friction of the molecule during the pulling process was extracted using the simple harmonic oscillator (SHO) model. Using this method we have studied the extension process of dextran. Dextran exhibits the characteristic force-extension profile due to the force-induced conformational change of the pyranose ring. However, recent MD studies suggest the existence of another intermediate state. In this study, we used smaller cantilever to reduce the friction comes from the cantilever. As a result of the improvement of the S/N ratio, a small shoulder was seen in the stiffness/friction versus force plots at around 200 pN. We consider that this shoulder reflects the conformational transition to the intermediate state predicted.

3P-248 単一分子ダイナミクスの実時間観察を可能にする2チャンネル同時タイムスタンプ計測

Dual time-stamp measurement for realtime observation of single molecular dynamics

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We developed a new microscope system based on a confocal microscope for single molecule fluorescence measurements. The microscope is equipped with two avalanche photodiode (APD) detectors and is enabled to obtain time stamp signals on both detector channels simultaneously. Instead of photon counting detection or imaging with a camera, in which photons are counted within a time bin or a frame with a fixed period, the time stamp records the detection time of every single photon. Since the time stamp signals contain entire information of the temporal distribution of photon emission, it can achieve the highest time resolution[1-3]. Two detector channels detect photons from the different wavelength regions spectrally separated by a dichroic filter and realize the fluorescence resonance energy transfer (FRET) measurement. The developed system is applied to FRET measurements of DNA and protein dynamics in single molecules.

[1] L. Watkins and H. Yang: *Biophys. J.*, 86, 4015, 2004

[2] L. Watkins and H. Yang: *J. Phys. Chem. B*, 109, 617, 2005

[3] K. Okamoto, M. Nishiyama and M. Terazima: 44th Annu. Mtg. BSJ, 2006.

3P-249 共焦点観察によるマウス側脳室繊毛のnm精度運動解析

Motility analysis with nm-accuracy and high temporal resolution of mice ependymal cilia by confocal imaging

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Motile cilia at lateral ventricle are required for circulation of cerebrospinal fluid. The ciliary movement had so far been observed by the differential interference contrast (DIC) observation, but there was a problem that could not obtain the slice