3P292 AFMによるグルタミン酸脱水素酵素結晶表面の観察
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Observation of the Surface of Glutamate Dehydrogenase's Crystals by Atomic Force Microscopy
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Correlations between protein motions and hydration changes are of quit importance for understanding protein function in aqueous solution. To investigate the correlations, we use complementary some experimental techniques and molecular dynamics (MD) simulations. Cryogenic X-ray crystallography provides space-time-averaged structures of hydrated protein molecules, and MD simulations do motions of hydrated protein molecules at high spatial and time-resolution in a short period. Then, experimental techniques are additionally necessary for compensating disadvantages in the above two methods. For this purpose, we are trying to apply atomic force microscopy (AFM) having a potential to detect structural changes of single protein molecules.

Here we show a series of AFM observation of the surface of glutamate dehydrogenase (GDH) crystal. GDH comprising six identical subunits with of Mw of 46k has a cylindrical shape with the height and the diameter of 10 nm. X-ray crystallography of GDH at a resolution of 0.18 nm revealed domains of the enzyme exhibited domain fluctuations about 1 nm even in crystalline state. In the AFM measurement, we found significant ruggedness on the surface of GDH crystals as high as 1 nm. The ruggedness is now under statistical analyses by gathering a lot of AFM images in assuming that it comes from the domain movement of GDH molecules on the surface.

3P294 クライオ原子間力顕微鏡による凍結割断試料の液浸観察
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Atomic force microscopy of freeze-fractured biological specimens in cryogenic solvent
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Atomic force microscopy (AFM) has been established as an important tool to analyze the surface topology of a biological specimen at a nanometer resolution. For observation of intercellular structures, it would be nice to combine the freeze-fracture-etching method and AFM. We have previously fabricated a cryogenic AFM that is able to analyze a surface of a freeze-fractured sample. In the previous studies, the freeze-fracture was performed by cleaving a frozen sample in cold nitrogen gas. Disadvantage of this procedure is that a frozen sample can't be fractured successively. In this study, we challenged to develop a novel method to obtain 3D images of a frozen sample. Namely, a frozen sample dipped in cold ethanol at -100°C was fractured using a knife and was then transferred to a cryogenic stage of AFM, by which the newly fractured surface is observed in cold ethanol. It is possible, in principle, to obtain 3D images of the frozen sample by repeating the above procedure. To investigate the performance of the newly developed technique, we observed vesicular structures of bacteriorhodopsin that were formed in a pre-crystallization solution. It was found that the average size of the vesicular assembly varied when a small amount of DMPC was added to the crystallization solution.

3P293 原子間力顕微鏡による超好熱菌および常温菌由来タンパク質の力学的安定性の計測
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Measurement of mechanical stability of proteins from extreme thermophile and mesophile with AFM
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Atomic force microscope (AFM) is capable of not only imaging biomolecules with nano meter spatial resolution but also measuring of mechanical properties such as extension length and force responsible for maintaining the 3D structure of protein. We have previously reported the measurements of mechanical properties of 3-isopropylmalate dehydrogenase (IPMDH), which forms a dimer of identical subunits and is responsible for biosynthesis of leucine, by AFM. In this study, we investigated the mechanical stability of IPMDH from extreme thermophile, *Thermus thermophilus*, and from mesophile, *Bacillus subtilis*, using AFM at high temperature (55 °C) to elucidate the relationships between the mechanical and the thermal stability of the protein. We found that at the high temperature, there were large differences in distribution of the maximum extension length between the IPMDH from extreme thermophile and mesophile (extreme thermophile, 10~45 nm; mesophile, 10~120 nm). On the other hand, our previous study showed that at the room temperature (25 °C), maximum extension length of IPMDH from extreme thermophile and mesophile were almost the same. These results indicate that at the high temperature, IPMDH from extreme thermophile has more stable structure than that from mesophile. In the presentation, we will also report the rupture force of the structure of IPMDH and discuss the structure-thermal stability relationship of the proteins based on these results. This work is supported by NEDO and Food Nanotechnology Project, MAFF.

3P295 AFMによる受容体とリガンドの相互作用観察
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Observation of interaction between ligand and receptor by AFM
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There are a lot of functionally important proteins such as receptors on cell membrane. The conventional optical microscopes, phase, DIC or fluorescent microscopes are used to obtain the image and analyze of proteins. However, as their resolution is about 300 nm, the analysis is difficult in nano-meter scale.

In this study, the CHO stably expressing the CFP tagged human lectin-like oxidized low-density lipoprotein receptor (CFP-LOX-1, established by S. Matsunaga et al) was investigated by atomic force microscope (AFM). The AFM instrument was mounted on a conventional inverse optical microscope.

AFM-silicon nitride probe was modified with an acetylated low-density lipoprotein from human plasma (AcLDL). The area of AFM-tip can be modified by physical adsorption. During modified AFM-probe approaching on the LOX-1-expressing cell, the cell reacted significantly. Therefore, the modified AFM-probe can be used for functional studies in nano-biology. Thus, AFM is a powerful tool for analyzing the structure and the function of cells with higher resolution than conventional optical microscopes. Our group is now investigating the qualitative analysis of the AFM image and the application of AFM for nano-biology. This study was supported by fund of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government, and supported by a grant from The New Energy and Industrial Technology Development Organization (NEDO), Japan.