【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Biology)

Title of Project : Opening up New Structural Biology by High-speed Atomic Force Microscopy

Toshio Ando
(Kanazawa University, School of Mathematics and Physics, Professor)

Research Area : Biophysics
Keyword : Structure, dynamics and functions of proteins and nucleic acids; Bioimaging

【Purpose and Background of the Research】

Since the function of proteins is tightly related to their structure, the detailed structure of proteins has been extensively studied but structures obtained are limited to static snapshots. The dynamic behavior of protein molecules in action has been studied by single-molecule techniques but the entities (protein molecules) are invisible. Thus, the simultaneous assessment of structure and dynamics has long been infeasible, meaning that we have to infer how proteins operate to function from gleaned data with significant resolution gaps. Therefore, directly visualizing functioning protein molecules at high spatial and temporal resolution has long been a "holy grail" for biological science. To overcome this longstanding problem, Ando has been developing high-speed atomic force microscopy (HS-AFM) since 1993, which has now reached its maturity. In fact, recent application studies conducted by Ando's group have continuously demonstrated that high-speed AFM is a powerful new approach to providing unique and deep insights into the functional mechanism of proteins (Fig.1). Moreover, it was recently demonstrated that even *in situ* dynamic visualization of protein molecules moving on live bacterial cell surfaces is also possible. Based on this innovative development of technology, this project aims at further expanding application studies and developing the next generation of microscopy techniques.

【Research Methods】

This project will perform the following studies. **(a)** HS-AFM imaging studies on various proteins will be performed through extensive collaborations with biologists: motor proteins, AAA proteins, DNA-related proteins, intrinsically disordered proteins, membrane transport proteins. **(b)** *In situ* video imaging will be carried out for outer surfaces of bacteria and isolated intracellular organelles (nuclei and mitochondria) to reveal dynamic molecular processes occurring thereon. **(c)** A non-contact type of high-speed scanning probe microscopy (SPM) will be developed to make it possible to visualize the surface structure of live eukaryotic cells. Moreover, developing new microscopy techniques will be attempted to make it possible to observe the interior of live cells at high spatiotemporal resolution.

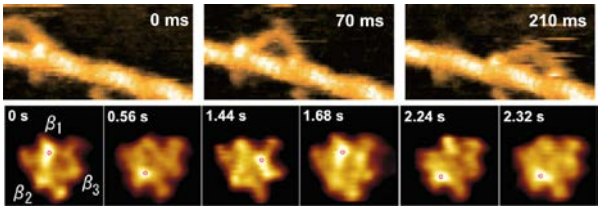

Figure 1 Proteins in action captured by HS-AFM. Upper panel: walking myosin V, Lower panel: rotary propagation of conformational change of rotorless F_1-ATPase.

【Expected Research Achievements and Scientific Significance】

Extensive successful demonstration of dynamic imaging of proteins in action will be achieved, which will innovate on the present condition of structural biology to open up "dynamic structural biology". The *in situ* imaging, together with new high-speed SPM techniques, will also bring a great impact to cell biology.

【Publications Relevant to the Project】

N. Kodera et al., "Video imaging of walking myosin V by high-speed atomic force microscopy", *Nature* **468**, 72-76 (2010).
T. Uchihashi et al., "High-speed atomic force microscopy reveals rotary catalysis of rotorless F_1-ATPase", *Science* **333**, 755-758 (2011).

【Term of Project】 FY2012-2016

【Budget Allocation】 165,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.s.kanazawa-u.ac.jp/phys/biophys/index.htm
tando@staff.kanazawa-u.ac.jp