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

Title of Project : Single-molecule Physiology by Assistance with a Soft Force

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Research Area : Biophysics
Keyword : Single-molecule measurements and manipulation; Structure, dynamics and functions of proteins and nucleic acids

【Purpose and Background of the Research】

A single protein molecule, a tiny entity mere millionth of a centimeter, performs a marvelous function(s) and hence is called a molecular machine. Examples include an ion channel that selects a particular species of ions and let them pass across a membrane in response to an electrical signal, and a rotary molecular motor F_1-ATPase. Mechanisms of these molecular machines can by studied by single-molecule physiology where one watches, and manipulates if needed, individual molecules at work under a microscope.

Manipulations have so far been mostly in the negative direction, obstructing or impeding the machine. Observations of natural or obstructed behaviors do not necessarily lead to an unequivocal interpretation. The best way to understand a complex system such as the lever escapement (Fig. 1) would be to "let it work" with your fingers, which also "feel" whether the move is right. We propose to do this on molecular machines. We deprive a molecular machine of its energy source, or delete its important part, and ask, instead of whether the machine fails, whether there is a way to let it work.

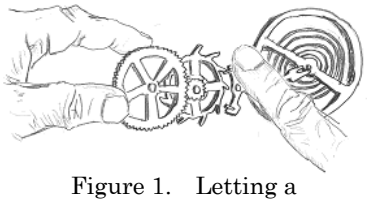

Figure 1. Letting a mechanical clock tick by

【Research Methods】

Our current goals are depicted in Fig. 2a-c.

A voltage-gated ion channel (Fig. 2a) is supposed to open when its voltage-sensor domains with multiple charges move in response to an applied voltage. Voltage, however, could exerts many effects on different parts of the channel and the membrane. We will directly pull the sensors to prove that their movement alone suffices, and inquire how much force is needed to open the channel.

F_1-ATPase, when alone, rotates by hydrolyzing ATP. In nature, it is joined to another motor F_o, which forcibly rotates F_1 in reverse, resulting in reversed hydrolysis, or ATP synthesis. To explore how, we replace F_o with magnets and apply a soft force for reverse rotation (Fig. 2b) to feel how F_1 reacts or how it may occasionally rotate on its own. We will also study reverse gyrase which winds up DNA double helix (Fig. 2c), probably for protection against thermal melting. We will twist DNA with magnets to assist, or obstruct, the enzyme.

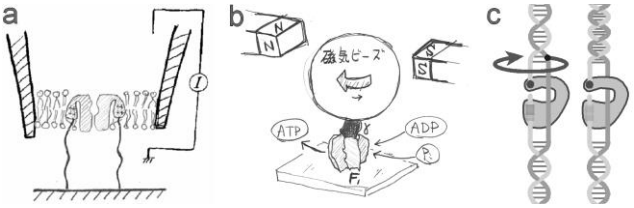

Figure 2. Letting protein machines work by a soft force.

【Expected Research Achievements and Scientific Significance】

It must be a formidable challenge to let a molecular machine work by hands. But the reward would be a decisive answer that this particular force or movement IS the causal key to the whole operation. We propose this high level of single-molecule physiology, which may also lead to the creation of new functions or new machines.

【Publications Relevant to the Project】

K. Adachi, K. Oiwa, M. Yoshida, T. Nishizaka, and K. Kinosita Jr. "Controlled rotation of the F_1-ATPase reveals differential and continuous binding changes for ATP synthesis" *Nat. Commun.* **3** (2012) 1022.

K. Yogo, T. Ogawa, M. Hayashi, Y. Harada, T. Nishizaka, and K. Kinosita Jr. "Direct observation of strand passage by DNA-topoisomerase and its limited processivity" *PLoS ONE* **7** (2012) e34920.

【Term of Project】 FY2014-2017

【Budget Allocation】 115,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.k2.phys.waseda.ac.jp