Giant acceleration of diffusion observed in a single-molecule experiment on F_1-ATPase

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I. ABSTRACT

The giant acceleration (GA) of diffusion is one of the modern diffusion theories in the field of non-equilibrium statistical mechanics, and was predicted by the theoretical analysis given in Ref. [P. Reimann *et al.*, Phys. Rev. Lett. 2001]. In this study, we applied the theory of the GA of diffusion to a single-molecule experiment on a rotary motor protein, F_1, which is a component of F_oF_1 adenosine triposphate (ATP)-synthase. The energetic properties of F_1 were discussed on the basis of the above mentioned theory and the high energy barrier of the rotary potential was estimated to be $20k_BT$ for the first time, with the condition that the adenosine diphosphates (ADPs) were tightly bound to the F_1 catalytic sites.

II. INTRODUCTION

The diffusion phenomena of microscopic particles, heat energy, electrons, etc., are common mechanisms that are routinely observed. They occur in solids, liquids, gases, and even in super-critical fluids, as a result of the thermal motion of the atoms and molecules which constitute these media. Many diffusion theories exist in the field of non-equilibrium statistical mechanics, one of which is the giant acceleration (GA) of diffusion [1-3]. According to Ref. [1], when a constant force is applied to a colloidal particle in a periodic potential, the diffusion of the particle is greatly enhanced. An increase of up to 14 orders of magnitude can occur, compared to free thermal diffusion for a realistic experimental setup. The diffusion coefficient as a function of an applied force exhibits a resonance peak at the force value near the critical tilt of the potential, which becomes increasingly pronounced as the environmental temperature decreases or as the energy barrier (Δ_E) of the periodic potential rises (Fig. 1(a)(b)). Previously, giant enhancement of the free thermal diffusion has been observed in an experiment on metal nanoparticles in the force field of an optical vortex lattice [4].

In this study, we present an effective application of the GA of diffusion to a single-molecule experiment on a rotary motor protein, F_1-ATPase (F_1), which is a component of F_oF_1-adenosine triphosphate (ATP) synthase [5].

The minimum complex in F_1 that can act as a motor is the $\alpha_3\beta_3\gamma$-subcomplex (Fig. 2(a)). When F_1 is isolated from F_o, the γ-subunit (rotor) rotates in the $\alpha_3\beta_3$-subunit (ring), hydrolyzing ATP into ADP (adenosine diphosphate) and Pi (phosphate). In a cell, F_1 is forced to rotate in the reverse direction by the F_o-motor to synthesize ATP from ADP and Pi. In order to enhance the efficiency of the ATP synthesis, F_1 has a mechanism, such as ADP inhibition, to inhibit the ATP hydrolysis caused by the spontaneous rotation of F_1 itself [6]. In fact, we often observe long pauses during a single-molecule assay during which F_1 falls into the ADP inhibition state. At this point, ADP is tightly attached to a catalytic site of F_1 and is not released.

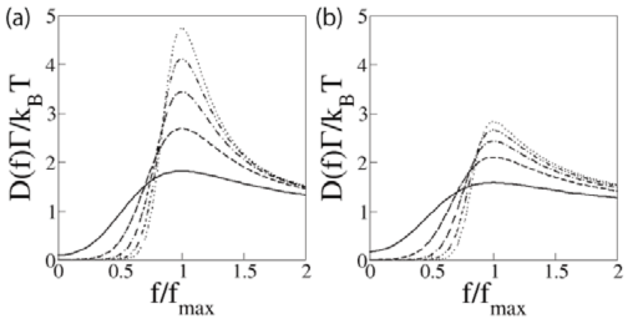

FIG.1. Theoretical results obtained in Ref. [1] and applied to our F_1 experiments. The dimensionless expression of the diffusion coefficient $D(f)\Gamma/k_BT$ as a function of f/f_{max} for (a) a sinusoidal potential and for (b) a triangle potential with the energy barrier height Δ_E=5, 10, 15, 20, and 25 k_BT (from bottom to top). f_{max} is the force at which D(f) reaches its peak.

In our study, the application of the GA of diffusion to F_1 made it possible to estimate the high energy barrier of the rotary potential for the first time, under the condition that the ADPs were tightly attached to the catalytic sites of F_1. We forced a rigid F_1 to rotate by applying an external torque using a single-molecule technique (Fig. 2(b)), and found that the diffusion coefficient of a probe attached to F_1 (as a function of the external torque) shows a resonance peak. The energy barrier was estimated from this peak and found to be approximately 20 k_BT at 25°C (where k_B is the Boltzmann constant and T is the environmental temperature).

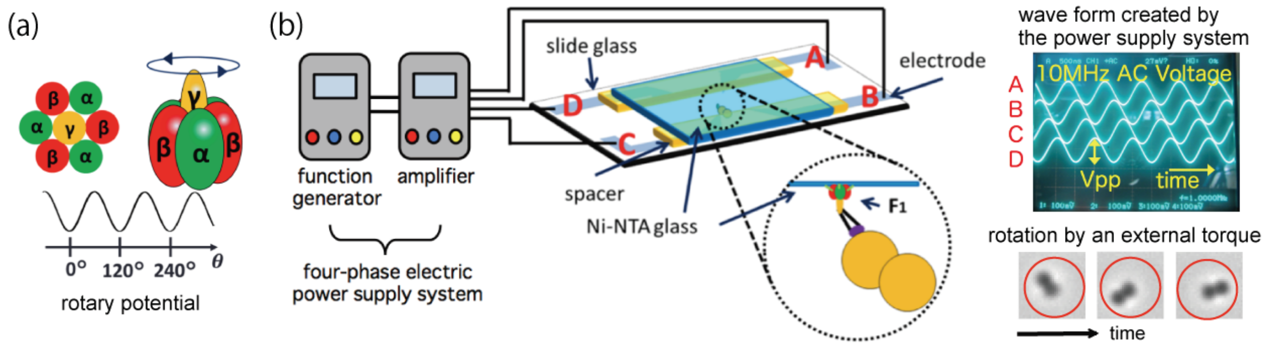

FIG. 2. (a) Because the structure of F_1 has three-fold symmetry,its rotary potential has a period of 120°. (b) Electro-rotation method [7]. A duplex of polystyrene beads (460-nm diameter), which is a dielectric, is attached to the γ-subunit of F_1. F_1 is fixed on the top glass surface (Ni-NTA-coated coverslip). At the center of the four electrodes, a rotating electric field with the frequency of 10 MHz is generated by applying sinusoidal voltages with a $\pi/2$ phase shift. The phase delay between the electric field and the dielectric moment of the duplex generates a constant torque, N_{ex}.

III. THE CONFERENCE GOAL

In the conference, we would like to offer the opportunity to discuss the way to extract energetic information from noise, which is useful for single-molecule experiments. Because bio-molecules work subject to thermal noise, the measurement method based on a noise analysis is significant for single-molecule experiments. A development of methods using a new theory on noise is surely an open problem.

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