Biochemical and Biophysical Research Communications 466 (2015) 196–200

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

H^+ and Na^+ are involved in flagellar rotation of the spirochete *Leptospira*

Md. Shafiqul Islam [a], Yusuke V. Morimoto [b, c], Seishi Kudo [a], Shuichi Nakamura [a, c, *]

[a] Department of Applied Physics, Graduate School of Engineering, Tohoku University, 6-6-05 Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[b] Quantitative Biology Center, RIKEN, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan
[c] Graduate School of Frontier BioSciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 28 August 2015
Accepted 1 September 2015
Available online 5 September 2015

Keywords:
Leptospira
Spirochete
Flagellar motor
Stator
Input energy

ABSTRACT

Leptospira is a spirochete possessing intracellular flagella. Each *Leptospira* flagellar filament is linked with a flagellar motor composed of a rotor and a dozen stators. For many bacterial species, it is known that the stator functions as an ion channel and that the ion flux through the stator is coupled with flagellar rotation. The coupling ion varies depending on the species; for example, H^+ is used in *Escherichia coli*, and Na^+ is used in *Vibrio* spp. to drive a polar flagellum. Although genetic and structural studies illustrated that the *Leptospira* flagellar motor also contains a stator, the coupling ion for flagellar rotation remains unknown. In the present study, we analyzed the motility of *Leptospira* under various pH values and salt concentrations. *Leptospira* cells displayed motility in acidic to alkaline pH. In the presence of a protonophore, the cells completely lost motility in acidic to neutral pH but displayed extremely slow movement under alkaline conditions. This result suggests that H^+ is a major coupling ion for flagellar rotation over a wide pH range; however, we also observed that the motility of *Leptospira* was significantly enhanced by the addition of Na^+, though it vigorously moved even under Na^+-free conditions. These results suggest that H^+ is preferentially used and that Na^+ is secondarily involved in flagellar rotation in *Leptospira*. The flexible ion selectivity in the flagellar system could be advantageous for *Leptospira* to survive in a wide range of environment.

1. Introduction

Leptospira is a spirochete distinguished by a coiled cell body and intracellular flagella. Pathogenic *Leptospira* strains are causative agents of the worldwide zoonosis leptospirosis [1]. The pathogenicity of *Leptospira* species is known to be correlated with their motility, and motility-deficient strains have a markedly decreased virulence [2]. *Leptospira* has two intracellular flagella that are present between the outer membrane and the peptidoglycan layer at both ends. Structural analysis using electron cryotomography revealed that each *Leptospira* flagellar filament is linked with a flagellar motor that is embedded in the cytoplasmic membrane at the end of the cell body [3]. Genome analysis revealed that the fundamental components of the *Leptospira* flagellar motor are similar to those of *Escherichia coli* [4]. It is believed that the rotation of the intracellular flagella transforms the cell body of *Leptospira*, rotating the spiral cell body and allowing the cell for translational motion [5].

The structure and function of flagellar motors have been studied in externally flagellated bacteria [6–8]. The flagellar motor consists of a rotor and stator. The rotor is surrounded by a dozen stator units that play the role of ion channels transporting specific ions from the cell exterior to its interior, and the ion flux is coupled with torque generation [6–8]. The coupling ion differs depending on the type of stator [9]. The stator of *E. coli* and *Salmonella* is formed by MotA and MotB proteins, and their flagellar rotations are coupled with H^+ influx through the MotA/MotB stator units [10,11]. The marine bacterium *Vibrio alginolyticus* has two types of flagella: multiple lateral flagella (Laf) and a single polar flagellum (Pof) [12,13]. Laf and Pof contain the H^+-type stator unit MotA/MotB and Na^+-type stator unit PomA/PomB, respectively [12]. Pof is used in low-viscosity environments, and the expression of Laf is triggered by an increase of viscosity [14–16]. *Shewanella oneidensis* has a single polar flagellum. However, both MotA/MotB and PomA/PomB are

* Corresponding author. Department of Applied Physics, Graduate School of Engineering, Tohoku University, 6-6-05 Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan.
E-mail address: naka@bp.apph.tohoku.ac.jp (S. Nakamura).

http://dx.doi.org/10.1016/j.bbrc.2015.09.004
0006-291X/

expressed, and the selection of one over the other depends on the environmental concentrations of Na^+; PomA/PomB is efficiently incorporated into the motor in the presence of high levels of Na^+, but it is exchanged with MotA/MotB when Na^+ concentrations are reduced [17]. *Bacillus subtilis* has the H^+-type MotA/MotB and Na^+-type MotP/MotS, and exchange of the stator is triggered by a change in the environmental pH [18].

The coupling ion driving movements of spirochetes has been studied in some species but information is limited. *Spirochete aurantia* [19] and *Borrelia burgdorferi* [20] are known to use H^+ for motility. It is predicted that H^+ is responsible for motility in *Leptospira* because homologs of *motA* and *motB* are present in its genome [4], but experimental evidence has not been obtained. In this study, we illustrate that both H^+ and Na^+ are involved in the motility of the saprophytic *Leptospira* strain *L. biflexa*.

2. Materials and methods

2.1. Bacterial strain and media

Cells of *L. biflexa* strain Patoc I were grown at 30 °C for four days in Ellinghausen–McCullough–Johnson–Harris (EMJH) liquid medium. Conventional EMJH growth medium contains several types of Na^+ and K^+ salts: 1 g of Na_2HPO_4, 0.3 g of KH_2PO_4, 1 g of NaCl, and 1.1 mg of sodium pyruvate per liter. In experiments testing the involvement of Na^+ or K^+ in motility, K^+- or Na^+-free EMJH medium in which all K^+ salts were replaced with Na^+ salts or vice versa was used to prevent contamination of ions from the growth medium to the motility medium. In motility assays, a motility medium for *Leptospira* designed by Lambert et al. [21] was prepared with some modifications, in which only 7 mM Na_2HPO_4 and 2.2 mM KH_2PO_4 were contained. 20 mM Tris–HCl was also used as a motility medium. The protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added to the motility medium if necessary.

2.2. Motility assay

Cells were diluted 1:100 in the motility medium. All motility assays were performed using a dark-field microscope (BH-2, Olympus, Japan). For measurements of swimming speeds, the free swimming of *L. biflexa* cells was recorded using a CCD camera (SSC-M350, Sony) at an interval of 30 ms and analyzed as described previously [22]. For measurements of cell-body rotation rates, cells were recorded using a high-speed camera (ICL-B0620M–KC, IMPERX) at an interval of 4 ms and analyzed as described previously [22]. All motility assays were conducted at room temperature.

2.3. Measurement of membrane voltage

The membrane voltage of cells was measured using the Nernstian fluorescent dye tetramethyl rhodamine methyl ester (TMRM), as described by Lo et al. [23] with some modifications. The 4-day culture was centrifuged at 800 × *g* for 5 min, and the sediment was suspended in 20 mM Tris–HCl (pH 7.5) containing 20 mM EDTA and 0.1 μM TMRM. The cell suspensions were incubated at 30 °C for 30 min with shaking and washed with Tris–HCl to remove EDTA. Next, 0.1 μM TMRM was added to the cell suspension before observation. Fluorescent cells spontaneously adhered on the glass surface were observed using the total internal reflection fluorescence microscope system as described previously [24]. TMRM was excited by a 150 mW Ar laser (514 nm, 35-LAL-515, Melles Griot), and emission was detected through a 610/75 nm filter (Chroma). The membrane voltage was calculated as described by Lo et al. [23] with minor modifications. To measure the membrane voltage under alkaline conditions, Tris–HCl with a pH value of 8.5 was used because the stability of cell adhesion on the glass was weak at pH 9.0, decreasing the accuracy of measurements.

3. Results

3.1. Classification of the motile form

The motile form of *Leptospira* spp. is diverse; some cells unidirectionally swim by rotating the cell body, and others simply rotate without translating [25,26]. We classified the motility of *L. biflexa* cells as "translation" and "rotation" (Fig. 1A). The translating cells exhibited a spiral shape at the anterior end and a hook shape at the posterior end (Fig. 1A, upper panel), and the rotating cells displayed hook shapes at both ends (Fig. 1A, lower panel). These observations are consistent with previous reports [25,26]. In the growth media, 24% and 68% of cells displayed translational and rotational motions, respectively (Fig 1B). In this study, we measured swimming speeds and cell-body rotation rates as the motile parameters of translating and rotating cells, respectively.

3.2. Motility under various pH conditions

Motility analysis has been performed only at a pH of approximately 7.5 [27] because *Leptospira* spp. are grown at a pH of 7.4–7.5 [28]. To verify how *L. biflexa* reacts to changes in external pH, we examined its motility under various pH conditions. The motility was maintained in acidic to alkaline pH, and swimming speeds of cells displaying the translational motion were gradually increased with increase in pH (Fig. 2A). Cell-body rotation rates of cells displaying the rotational motion also increased with an elevation of pH (Fig. 2B).

3.3. Effect of CCCP on motility

We tested whether H^+ is a coupling ion of the *L. biflexa* flagellar motor. The driving force to transfer H^+ ions through stator units is an electrochemical potential difference of H^+ called the proton motive force (PMF) [6–8]. As PMF is corrupted by CCCP, H^+-driven

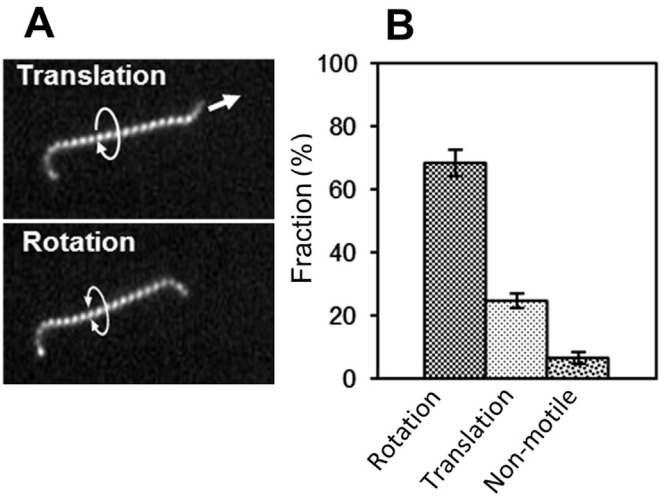

Fig. 1. Motile forms of *Leptospira biflexa*. (A) Microscopic images of motile cells. In the translating form (upper panel), cells move toward the direction indicated by the arrow with rotating their cell bodies. The lower panel shows a cell categorized into the rotating group. (B) Fractions of motile forms observed in EMJH growth media (pH 7.5). The average values of triplicate experiments are shown. More than 200 cells were analyzed in each experiment. Vertical lines denote the standard deviation.