EDITOR’S CHOICE

Viscosity-dependent variations in the cell shape and swimming manner of Leptospira

Kyosuke Takabe, Hajime Tahara, Md. Shafiqul Islam,† Samia Affroze, Seishi Kudo and Shuichi Nakamura*

Abstract

Spirochaetes are spiral or flat-wave–shaped Gram-negative bacteria that have periplasmic flagella between the peptidoglycan layer and outer membrane. Rotation of the periplasmic flagella transforms the cell body shape periodically, allowing the cell to swim in aqueous environments. Because the virulence of motility-deficient mutants of pathogenic species is drastically attenuated, motility is thought to be an essential virulence factor in spirochaetes. However, it remains unknown how motility practically contributes to the infection process. We show here that the cell body configuration and motility of the zoonotic spirochaete Leptospira changes depending on the viscosity of the medium. Leptospira swim and reverse the swimming direction by transforming the cell body. Motility analysis showed that the frequency of cell shape transformation was increased by increasing the viscosity of the medium. The increased cell body transformation induced highly frequent reversal of the swimming direction. A simple kinetic model based on the experimental results shows that the viscosity-induced increase in reversal limits cell migration, resulting in the accumulation of cells in high-viscosity regions. This behaviour could facilitate the colonization of the spirochaete on host tissues covered with mucosa.

INTRODUCTION

Spirochaetes are distinguished from other bacterial species by their spiral or flat-wave–shaped cell body and intracellular periplasmic flagella (PFs). Spirochaete PFs are present between the peptidoglycan layer and outer membrane and are linked to a rotary nanomachine flagellar motor embedded in the cytoplasmic membrane at both ends of the cell. The PFs play a cytoskeleton-like role in retaining cell shape, and their rotation periodically transforms the cell body for propulsion [1–3].

Many spirochaetes are human and animal pathogens, including Borrelia burgdorferi (Lyme disease), Treponema pallidum (syphilis), Brachyspira hyodysenteriae (swine dysentery) and Leptospira interrogans (leptospirosis). A strong correlation between motility and virulence is known in such pathogenic species [4–6]. Histological studies have shown that the intestinal spirochaetes Brachyspira aalborgi and Brachyspira pilosicoli adhere to the epithelium surface by penetrating the mucosa layer at one end of the cell body [7, 8]. The motility efficiency of Brachyspira pilosicoli is increased by adding the polymer polyvinylpyrrolidone to the medium [9]. Treponema denticola, the spirochaete involved in periodontal disease, can swim only in medium containing viscous agents [10]. In Leptospira spp., the spirochaete maintains swimming at high speed even in solutions containing methylcellulose (MC) [11, 12]. The pathogenic Leptospira strains, in fact, appear to favour higher viscosity, penetrating the animal body via mucous membranes and then adhering to host tissues [13]. Thus, active motility in highly viscous environments plays a key role in the spirochaete life; however, how the motility contributes to their colonization and infection is unclear.

Leptospira have two PFs within a short-pitch coiled cell body. These PFs extend from both ends toward a central region of the cell body but are not long enough to contact each other [14]. The ends of Leptospira cells are bent by PFs into a long-pitch spiral (S) shape or flat half-circle hook (H) shape (Fig. 1). The shape of the cell ends frequently changes between the S shape and the H shape. The asymmetric SH form, in which the anterior and posterior ends display...
S shape and H shape, respectively, allows the cell to swim (Fig. 1 upper panel). When displaying symmetric configurations (HH or SS), the cell does not migrate forward or backward but instead rotates one position (Fig. 1 middle and lower panels) [15–17].

In this study, we show the viscosity-dependent changes in cell body configuration and motility mode of *Leptospira*. We analysed the cell shape and motility of *Leptospira* in liquids containing various concentrations of MC and found that frequencies of cell shape transformation and swimming reversal were dependent on viscosity.

**METHODS**

**Bacterial strain and media**

The non-pathogenic species *Leptospira biflexa* strain Patoc1 was used. Cells were grown in Ellinghausen–McCullough–Johnson–Harris liquid medium supplemented with 10% BSA at 30 °C until mid-exponential phase (optical density at 420 nm, ca. 0.3).

Potassium phosphate buffer (20 mM; pH 7.6) was used as a motility medium, and the viscosity of the motility medium was increased by the addition of MC (Sigma). The viscosity was measured with a tuning-fork-type viscometer (SV-1A; A&D), giving the following values: no MC, 0.9 mPa·s; 0.25% MC, 2.3 mPa·s; 0.5% MC, 7.0 mPa·s; 0.75% MC, 16.7 mPa·s; 1% MC, 32.5 mPa·s.

**Microscopy and analysis for leptospiral motion**

The cells were diluted 1:20 into motility media and observed using a dark-field microscope (BX53, 40× objective, 2.5× relay lens; Olympus). The motions of the cells were recorded with a high-speed charge-coupled device camera (IMPERX) at a rate of 200 frames per second (exposure time ca. 5 ms). For measurements of swimming speeds, a speed versus time trace for individual cells was obtained by determining the cell centroid for every 5 ms; the entire speed–time trace was smoothed by moving average with 15-frame window; the maximum speed value in each trace was adopted as the swimming speed of the cell. We analysed the swimming trajectory, the cell shape transition and swimming speed using ImageJ software (National Institutes of Health) and a VBA macro originally developed in Microsoft Excel. All experiments were performed at 24 °C.

**RESULTS**

**Switching of the cell shape and motility mode**

We observed that the cell body configuration of *Leptospira* changed between the asymmetric SH shape and the symmetric HH or SS shape as represented in Fig. 1, allowing the cells to switch the motion mode between swimming mode and rotation mode, in agreement with previous reports [16, 18]. The movements of *Leptospira* are translational, as shown in Fig. 2(a). However, when transformation occurs at both ends of the cell body, the cell reverses swimming direction by 180° (Fig. 2b right panel). To discriminate between the SH and HS configurations in each cell (the S-end is side anterior to the swimming direction), we defined the swimming toward the end point as ‘forward’ and swimming back toward the start point of the trajectory as ‘backward’ movements (Fig. 2a). We regarded the configuration for forward movement as SH and that for the backward movement as HS (Fig. 2b).

**Time record of cell shape changes**

Using the definitions described above, we show the cell shape versus time plot obtained from experiments performed under low- and high-viscosity conditions. In the medium without polymer, the cell often exhibits the rotation forms (SS or HH), but its configuration is biased toward the SH form (Fig. 3a upper panel). In the high-viscosity medium containing 1% MC, the cell shape changes frequently between SH and HS via SS or HH (Fig. 3a lower panel). The frequency of transformation between cell configurations increased with viscosity (Fig. 3b).

**Swimming reversal**

Transformation of the cell shape from SH to HS via rotation modes (SH-SS-HS or SH-HH-HS) (Fig. 3a lower panel) indicates that the cell reverses swimming direction by 180° (Fig. 2b right). In contrast, the transformation of SH-SS (HH)-SH slightly changes the long-axis direction of the cell body but does not change the swimming direction of the cell (Fig. 2b left). We designated the former ‘reversal motion’ and the latter ‘stepping motion,’ and we quantified these events.

The swimming trajectories of individual cells are shown in Fig. 4(a), where the starting point of each trajectory is rearranged to the origin. Temporal records of the swimming distance obtained from the swimming trajectories are shown in Fig. 4(b). Typical stepping and reversal events observed in the
trajectories are extended in Fig. 4(c), clearly showing forward swimming by the SH-shaped cells (highlighted in red) and backward swimming by the HS-shaped cells (highlighted in blue). The rotation period (highlighted in green; SS and HH are not discriminated) is bounded by swimming periods, appearing as if the cell pauses.

Fig. 4(a) shows that the cells moved a greater distance in the medium without MC than in 1 % MC solution. Swimming speeds of cells in the medium without MC and in 1 % MC solution were $20.6 \pm 3.1 \mu m \ s^{-1}$ ($n=20$ cells) and $18.3 \pm 3.1 \mu m \ s^{-1}$ ($n=20$ cells), respectively (Fig. 4d). This result indicates that MC does not impair the Leptospira swimming unlike a branched polymer, Ficoll [9] (see Discussion). We examined the fraction of the stepping and reversal movements under various viscosity conditions. In low-viscosity medium, stepping dominated the Leptospira motion (Fig. 4e). As the viscosity increased, the fraction of reversal increased up to that of stepping motion (Fig. 4e). These results indicate that the net migration distance of individual cells is limited by the increased swimming reversal. Thus, Leptospira cells swim at the same speed in viscous and non-viscous media but are frequently moved backward by the reversal events in viscous media (Fig. 4a, b).
Fig. 4. Viscosity dependence of swimming pattern. (a) Swimming trajectories of 19 cells over the course of 5 s are shown in different colours. The starting point of each trajectory is relocated to the origin. (b) Time course of displacement obtained from trajectory data as shown in (a). (c) Examples of stepping and reversal movements (see the main text) extracted from (b). Asterisks (*) indicate expanded views presented in insets. Red, forward with the shape of SH; blue, backward with the shape of HS; and green, rotation with the shape of HH or SS. (d) Swimming speeds measured at various MC concentrations. Mean values and standard deviations of more than 20 cells are indicated. Some fraction of the observed cells showed continuous rotation without swimming; therefore, the cells moving either forward or backward were analysed. (e) Fraction of the stepping and reversal movements. The number of stepping ($n_{step}$) and reversal ($n_{rev}$) events observed in each cell trajectory was counted, and the ratio of the stepping and reversal movements was calculated: $n_{step}/(n_{step}+n_{rev})$ and $n_{rev}/(n_{step}+n_{rev})$. 

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**Kinetic characterization**

We determined the time period that the cells remained in the SH and HS forms from the cell shape versus time plot and then computed the time fractions. Fig. 5(a) is the ratio of time fraction of HS to that of SH ([HS]/[SH]), showing that the fraction of backward movement increased with viscosity in agreement with Fig. 4(c, e). The value of [HS]/[SH] corresponds to the equilibrium constants of the reaction from SH to HS ($K_{\text{sh-hs}}$). Because direct transformation between SH and HS was not observed in the present experiment, we assumed a simple kinetic diagram depicted in Fig. 5(b) and determined the equilibrium constant of each reaction from the time fraction of each mode: [SH], [HH], [SS] and [HS]. The left panel of Fig. 5(c) shows that $K_{\text{hh-sh}}$ decreases with increasing viscosity, whereas $K_{\text{hh-hs}}$ is almost constant. These indicate that, in the reactions via HH, suppression of transition from HH to SH energetically biases the reaction toward the reverse motion in high viscosity. The right panel of Fig. 5(c) shows that, in the reactions via SS, a clear increment of $K_{\text{ss-hs}}$ seems to increase the reversing probability. These results suggest a distinct response to viscosity between S-end and H-end.

**DISCUSSION**

In this study, we observed that *Leptospira* swam in a relatively smooth manner in low-viscosity media, but the frequency of cell shape transformation and swimming reversal increased with increasing viscosity. The shape transformation in *Leptospira* is believed to occur when the rotational direction of the flagella is switched between counterclockwise and clockwise [19]. The main cause to increase the flagellar switching frequency is the chemotaxis signalling pathway: stimulation of the chemotaxis receptors is followed by phosphorylation of the response regulator CheY-P then associates with the rotor of the flagellar motor, and the motor changes its rotation direction from counterclockwise to clockwise direction [20]. Although the cell shape transformation of *Leptospira* is related to chemotaxis [17], we confirmed that the increased cell body transformation by increasing viscosity was retained overnight (data not shown). This indicates that the cell did not adapt to the high viscosity, unlike its response to chemotaxis. Thus, although the leptospiral strain used in our experiment possesses an intact chemotaxis system, the observed increase in transformation frequency was most unrelated to chemotactic stimuli and was probably caused at a steady level of CheY-P. In the external flagella of *Escherichia coli*, the switching frequency is reported to change depending on viscosity, even in the absence of chemical stimuli [21, 22]. This phenomenon is distinct from conventional chemotaxis and is explained as a stochastic matter using a mathematical model that involves coupling of a conformational change in the rotor protein FliG by the unstimulated level of CheY-P with torque generation [23]. Although the *Leptospira* flagellar motor can be distinguished from that of *E. coli* by additional structures, e.g. the periphery of the C-ring [24], it possesses components contained in most flagellar motors in common, including FliG and the stator proteins [25]. Thus, the viscosity-dependent phenomena observed can be interpreted as it was in the case of *E. coli*.

How does increased viscosity induce the reversal motion? Regarding the spirochaete motility in polymer solutions, it is known that swimming speeds of *Borrelia* [26] and *Brachyspira* [9] are decreased by the addition of Ficoll to media, which is a Newtonian fluid; whereas, in the viscoelastic MC or polyvinylpyrrolidone solution, *Borrelia* swim faster [27], and *Brachyspira* do not decrease the swimming speed even at high polymer concentrations [9]. As with these bacteria, the swimming of *Leptospira* is known to be slowed by the addition of Ficoll [18], but Fig. 4(d) shows that the...
swimming speed of *Leptospira* does not decrease with increases in MC concentration. These data indicate different involvement of viscosity and elasticity in motility; namely, viscosity is a drag on the cell movement and elasticity could allow the cell to swim without slippage [16]. We suspect that the viscoelastic property of MC is responsible for the increase in reversal frequency in high-viscosity solutions. However, we also observed viscosity-dependent changes in shape transformation frequency and reversal fraction in Ficoll solution (Fig. S1, available in the online Supplementary Material). Therefore, the elastic property of the polymer solution might not be a major factor in increasing reversal event; rather, the viscosity itself possibly causes the phenomenon as a physical stimulus. Studies to improve our understanding of the interaction between bacteria and their fluid environment are necessary.

The limitation in migration caused by an increase in reversal events in highly viscous environments seems to be disadvantageous for a species that live parasitically in host animals. Is there any biological significance to this behaviour? *Leptospira* are known to accumulate in regions of high viscosity [28]. While the precise mechanism of this behaviour, proposed as 'viscotaxis,' remains unclear, it is thought that an increased swimming velocity in response to the increased viscosity is responsible for the taxis-like behaviour [28]. We would like to propose another plausible model based of the concept that cells entering an area of high viscosity are ‘trapped’ by the impaired migration. To verify this hypothesis, we computationally demonstrate the cell behaviour in a virtual viscous fluid using a simple kinetic model (Fig. 6a). The computer simulation was performed with the following assumptions (see also Fig. 6 legend):

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**Fig. 6.** Simulation of motility-form switching depending on viscosity. (a) A simple kinetic model. The changes in motility form at the rate constants (*k*) are indicated in the diagram (left). 1D movements of cells were simulated (right). The rate constants were changed depending on the viscosity condition of each cell position: The experimental values obtained in the medium without MC and with 1 % MC were used for regions of low viscosity and high viscosity, coloured, grey (see also the main text). All values of the rate constant are listed in Table S1. (b) Results of the position–time plots for every cell, obtained by simulation under the condition of uniformly low viscosity (upper panel), uniformly high viscosity (middle panel) and a viscosity gradient (lower panel). The right panels show three typical traces extracted from the left panels. (c) Model of *in vivo* bacteria colonization. Red dots are chemically attractive substrates. Two types of arrows represent the straight swimming with stepping and reversal movement.
(1) Cells (n=10) move in a one-directional space with or without a high-viscosity area.

(2) The equilibrium constants are dependent on viscosity as shown in Fig. 5(c). In the low viscosity (motility medium without MC in the experiment), the stepping motion dominates the cell behaviour: the cell movements are biased toward the forward direction. The reversal motion increases when the cell enters the high-viscosity area (1% MC in the experiment; coloured light grey in Fig. 6).

(3) The cell shape change occurs following the rate constants k (e.g. \(k_{hh} \rightarrow sh\)) is the rate constant for the transition from HH to SH). The values of \(k_{hh} \rightarrow sh\), \(k_{hh} \rightarrow ss\) and \(k_{ss} \rightarrow ha\) were determined as reciprocals of the duration times for HH before SH, HH before HS, SS before SH and SS before HS, respectively, acquired from the cell shape versus time plot (Fig. 3a); the counterpart rate constants in each transition were calculated by using the equilibrium constants shown in Fig. 5(c), e.g. \(k_{hh} \rightarrow hh\). The transition probabilities \(P\) are given as, e.g. \(P_{hh} \rightarrow sh\), \(k_{hh} \rightarrow sh\), \(\Delta t\), where \(\Delta t\) is the time interval of events (5 ms). The occurrence of the transition is determined by a random number (Rnd) from 0.0 to 1.0 generated for each cell in every event; if the cell is in the HH form and \(P_{hh} \rightarrow sh > Rnd\), the cell changes to the SH form.

Fig. 6(b) shows typical results of the simulation. Under the low-viscosity condition, the cell movements were biased toward forward migration (Fig. 6b top), which is consistent with the experimental result shown in Fig. 4. When the highly viscous region was uniformly assumed, corresponding to the experiment in 1% MC, the elongated reversal movements and cells hovering without migration were observed (Fig. 6b middle), in agreement with Fig. 4. When a region with high viscosity was assumed to be present within the low-viscosity region, the movements of the cells entering the region of high viscosity were limited, resulting in accumulation (Fig. 6b bottom). The reversal movement was enhanced by the addition of mucus to media (Fig. S2); mucus is a major viscous agent for animal tissues and is known to attract Brachyspira pilosicoli [29]. Furthermore, we have confirmed that the pathogenic strain Leptospira interrogans reversed more frequently in high viscosity (Fig. S3). Taken together with these experimental results and the fact that chemotaxis and motility are crucial factors for spirochete infection [5, 6, 30], our model implies that the colonization of bacteria in vivo, as in host animals and natural environments, would be facilitated by a combination of chemical-attractive substrates and viscosity, as shown in Fig. 6(c) (colonization on the epithelium is depicted as an example) and as follows: (1) chemotaxis allows bacteria to migrate near the tissue surface surrounded by the mucosa, (2) the reversal movement is enhanced by viscosity and (3) the net migration is impaired, assisting in adhesion to the tissue. Our simulation did not refer to the time dependency of the cell distribution: the cells will diffuse over a long time period; as a consequence, the cell distribution would be uniform even in the presence of the high-viscosity region. Thus, the impairment of cell migration by viscosity would be effective only for a limited period of time. Although the actual in vivo circumstance is surely more complex due to the jagged structure of organs and the flow of solid and liquid matter in organic tracts, temporary cell accumulation induced by viscosity would contribute to the initial step of bacterial colonization.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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