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Implications of coordinated cell-body rotations for *Leptospira* motility

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ABSTRACT

The spirochete *Leptospira* has a coiled cell body and two periplasmic flagella (PFs) that reside beneath the outer sheath. PFs extend from each end of the cell body and are attached to the right-handed spiral protoplasmic cylinder (PC) via a connection with the flagellar motor embedded in the inner membrane. PFs bend each end of the cell body into left-handed spiral (S) or planar hook (H) shapes, allowing leptospiral cells to swim using combined anterior S-end and posterior H-end gyrations with PC rotations. As a plausible mechanism for motility, S- and H-end gyrations by PFs and PC rotations by PF counter-torque imply mutual influences among the three parts. Here we show a correlation between H-end gyration and PC rotation from the time records of rotation rates and rotational directions of individual swimming cells. We then qualitatively explain the observed correlation using a simple rotation model based on the measurements of motility and intracellular arrangements of PFs revealed by cryo-electron microscopy and electron cryotomography.

1. Introduction

*Leptospira*is a member of spirochetes comprising the saprophyte strains and causative agents of a worldwide zoonosis, leptospirosis [1]. *Leptospira* have two periplasmic flagella (PFs) between the outer sheath (OS) and protoplasmic cylinder (PC). PFs reside at each end of the cell body and are connected with the flagellar motor embedded in the inner membrane. Unlike other spirochetes, such as *Borrelia* and *Brachyspira*, PFs of *Leptospira* are not long enough to contact each other at the center of the cell body [2]. Movement of *Leptospira* is slowed in homogeneously viscous media like a solution containing Ficoll, whereas the spirochete can vigorously move in structured viscoelastic media like polyvinylpyrrolidone or methylcellulose solution [3,4]. The motility of *Leptospira* is a crucial virulence factor [5,6].

Leptospiral cells show right-handed coiled configurations over the entire cell body [7,8], and both ends of the cell body bend into either left-handed spiral (S) or planar hooked (H) shapes. Mutant *Leptospira* that lack PFs also lack the bent end, indicating that PFs act as the cytoskeleton to form the S-end and H-end [2]. Moreover, it is believed that rotational directions of PFs determine whether the cell ends will form S-shape or H-shape [7,9,10]. Both ends of the cell body switch between S-shape and H-shape during swimming, and thereby the cells show three distinct configurations S–H, H–H, and S–S, which are all associated with the motile form; S–H cells translate and S–S and H–H cells rotate without forward or backward translation [7,11]. When the cells displaying S–S or H–H form, the ends of the cells gyrate in opposite directions, while the middle of the cells remain stationary [9].

Although it remains unclear how the S-end, PC, and H-end are rotated by PFs, an approximate mechanism was previously derived from the cell structure and involved S- and H-end gyrations by PFs and PC rotation by countertorques of PFs. Motility assays and theoretical studies [7,11–13] have proposed that clockwise (CW; as viewing the cell body from the posterior H-end to the anterior S-end of a swimming cell) rotation of the right-handed PC and counterclockwise (CCW) gyration of the left-handed S-end are responsible for leptospiral swimming. The planar half-circle H-end was not associated with thrust generation in previous studies and rotated in the same direction as the S-end but with frequent CW turns [7,13]. Although the connection of both PFs with the PC suggests mutual influences among the three observed rotations, these have not yet been demonstrated in experiments.

In this study, we obtained time records of speeds and directions of S-end and H-end gyrations, and PC rotation in individual

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swimming leptospiral cells. Subsequent cross-correlation analyses showed clear associations between the H-end and PC, and we proposed a qualitative model for cell-body rotations in *Leptospira* according to motility assays and cryo-electron microscopy (cryo-EM) and electron cryotomography (ECT) analyses.

2. Materials and methods

2.1. Bacterial strain and media

Cells of the saprophyte *Leptospira biflexa* strain PatocI were grown at 30°C for 2–4 days in Ellinghausen–McCullough–Johnson–Harris (EMJH) liquid medium supplemented with 10% bovine serum albumin. Korthof's medium (pH7.4) containing 5% (w/v) Ficoll (Sigma-Aldridge, St. Louis, MO; viscosity, 2.5 mPa × s) was used as motility medium; proportions of cells swimming smoothly in the S–H configuration were increased by adding 5% Ficoll to the medium [14].

2.2. Motility analysis

Cells were observed using a dark-field microscope (BX53; Olympus, Tokyo, Japan) equipped with a 100 × oil immersion objective (UPlanFLN, Olympus, Tokyo, Japan) and a 5 × relay lens. The numerical aperture (NA) of the objective was adjustable (0.6–1.3) and the maximum in a range ensuring dark-field observation was used. Motions of individual cells were recorded at 500 frames per second using a high-speed CMOS camera (IDP-Express R2000, Photron, Tokyo, Japan). The recorded videos were captured in a computer and analyzed using ImageJ (National Institutes of Health, Rockville, MD) and a custom-made program that was developed using LabVIEW (National instruments, Austin, TX). Cross-correlation analyses were performed using the following formula [15]:

$$Z(\tau)=\frac{\frac{1}{N}\sum_{t=1}^{N-\tau}\left(x(t)-\overline{x(t)}\right)\left(y(t+\tau)-\overline{y(t+\tau)}\right)}{\sqrt{\frac{1}{N}\sum_{t=1}^{N-\tau}\left(x(t)-\overline{x(t)}\right)^2}\times\sqrt{\frac{1}{N}\sum_{t=1}^{N-\tau}\left(y(t+\tau)-\overline{y(t+\tau)}\right)^2}}, \quad (1)$$

Where τ is the delay time, t is time (= 1, 2, 3, …, N), N is the total number of data points, and x (t) and y (t) are time courses of rotation rates.

2.3. Cryo-EM and ECT

Sample solutions of 2.6 μL were applied to R0.6/1.0 Quantifoil grids (Quantifoil Micro Tools, Jena, Germany) glow-discharged in a weak vacuum for 20 s immediately before use. Grids were then blotted briefly with filter paper and rapidly plunged into liquid ethane using Vitrobot Mark II (FEI Company, Eindhoven, Netherlands). Cryo-EM images and ECT were collected at liquid-nitrogen temperature using Titan Krios electron microscope (FEI Company, Eindhoven, The Netherlands) equipped with a field emission gun and a Falcon direct electron detector (FEI Company, Eindhoven, The Netherlands). The microscope was operated at 300 kV and a nominal magnification of 29,000 × and 22,500 × with a calibrated pixel size of 5.71 and 7.6 Å.

Images of single-axis tilt series were collected covering an angular range from −60° to +60°with a nonlinear Saxton tilt scheme at 6 μm under focus using the Xplore 3D software package (FEI) and a cumulative dose of ~100 $e^-/Å^2$. The IMOD package [16] was used to align tilted projection images and to generate the final 3D density map from the aligned image stack. The final 3D density map was obtained by simultaneous iterative reconstruction technique (SIRT).

3. Results and discussion

3.1. Rotation analysis of S-end, H-end and PC

We acquired shallow-depth images of cell bodies using a high magnification objective and NA (see Materials and Methods) to observe coiled cell bodies as sequential bright spots (Fig. 1A). Because the PC is a right-handed helix, we determined its rotational direction from movements of the spots (moving backward by CW rotation). Directions of S-end and H-end gyrations were determined from the angle of each bright spot, which was dependent on the position of the focal plane relative to the cell-body axis (Fig. 1A and B). To determine gyration rates of the S-end and H-end, the curvature of a curve fitted to each cell end was measured (Fig. 1B, left and right panels). Gyration rates of the S-end and H-end were determined for every revolution from peak intervals of periodic changes in curvature (Fig. 1C, left and right panels). The rotation rate of PC was determined from changes in brightness in a fixed area (blue squares in middle columns of Fig. 1B) as previously described (Fig. 1C, middle panel) [13].

3.2. Time records of cell-body rotations

Fig. 2A shows typical examples of speed-time traces of S-end and H-end gyrations, and PC rotation. In agreement with previous reports, the S-end and H-end mostly gyrated CCW [7,9]. Gyration rates of the S-end and H-end considerably fluctuated, with standard deviations of 42% and 36% relative to the mean rate, respectively. The direction of H-end gyration sometimes switched from CCW to CW (Fig. 2A). The PC rotated CW at a frequency of approximately 160 Hz and the rotation was relatively stable, with a fluctuation ratio of 16%, as previously shown [13]. The right panels of Fig. 2A show relative rotation rates from the traces in the left panels, indicating differences in speed variations among the three rotations. Relative rotation rate–time traces show that speed variations of the PC appears to be almost coincident with those of the H-end, with brief speed reductions (shaded areas in the right panels of Fig. 2A).

3.3. Correlation analyses

Both S-end and H-end gyrations may influence PC rotation, because both of the PFs that reside at the S-end and H-end attach to the PC. However, the speed-time traces (Fig. 2A) suggest that PC rotation seems to be more closely connected with H-end gyration. Accordingly, averaged cross-correlation profiles (n = 21 cells) showed clear correlations between H-end and PC (Fig. 2B). Because PC rotation and H-end gyration are opposing (Fig. 2A), the negative value of the peak in the correlation profile indicates that if either accelerates, the other also accelerates. The peak position in the correlation profile indicates a delay time of PC rotation relative to H-end gyration; positive values indicate that speed variations of H-end preceded those of PC. The averaged profile indicates a peak at almost 0 ms (Fig. 2B), whereas the delay times varied between the expanded examples shown in Fig. 2C. These data suggest that H-end and PC influence each other.

Fig. 2B shows no correlation between S-end and PC, but weak correlation is observed between S-end and H-end. Although both S-end and H-end predominantly gyrate CCW, some cells showed that acceleration of S-end gyrations are transiently coincident with deceleration of H-end gyrations (Supplementary Fig. 1); consequently, the negative peak appears.

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