Reversal of Flagellar Rotation in Monotrichous and Peritrichous Bacteria: Generation of Changes in Direction

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Received for publication 20 May 1974

Reversal of flagellar rotation can explain both the “backing up” of monoflagellated Pseudomonas citronellolis and the tumbling of multiflagellated Salmonella typhimurium. Reversals occur spontaneously and can be induced by negative gradients of attractant and by high-intensity light.

The propulsion of bacteria has been identified with the helical motion of its flagella (3, 4, 10, 11). The suggestion of Stocker (10) and Doetsch (4) that helical motion involves rotation of a rigid propeller-like flagellum has been given strong support by the theoretical evaluation of Berg and Anderson (1a) and the experimental studies of Silverman and Simon (9). By the use of a high-intensity light source, Macnab and Koshland (6) were able to study the flagellar bundle of the peritrichously flagellated Salmonella typhimurium and observe that the movement is consistent with a left-handed helix rotating in a counterclockwise direction. Moreover, the high-intensity light allowed observation of individual flagella and led to the conclusion that tumbling occurs concomitantly with a flying apart of the flagellar bundle (6). Since bacteria respond to chemical gradients by regulating their tumbling frequency (2, 5), it was of interest to distinguish among three mechanisms for tumbling: (i) some flagella cease to function, (ii) the direction of the thrust changes, and (iii) the direction of rotation of the flagella is reversed (6). Studies on the monoflagellated Pseudomonas citronellolis (8) were initiated to distinguish between these alternatives. Recently, studies by Simon, Berg, Adler, and their colleagues (1, 4a, 9a) have indicated that reversal of flagellar rotation is involved in tumbling of the peritrichous bacteria and their detection of gradients. Our studies agree with that conclusion.

The motility pattern for P. citronellolis (Fig. 1) consists of runs in a forward direction, followed by a brief reversal of direction, and then another run in the forward direction. The cells do not tumble like S. typhimurium and Escherichia coli, but the reversals cause a reorientation of the bacteria, resulting in a random-walk type of behavior. After a sudden increase in attractant (serine) concentration, P. citronellolis underwent a period of smooth swimming in which changes in direction were suppressed, just as tumbling is suppressed in the peritrichous bacteria (5). When the Pseudomonas experienced a decrease in attractant (or increase in repellent) concentration, most cells oscillated rapidly between forward and reverse swimming (a “two steps forward, one step backwards” type of movement). Some cells stopped at frequent intervals instead of reversing. Intense blue light also caused frequent reversing or stopping.

The sense of direction of the Pseudomonas flagella appears to be that of a left-handed helix rotating in a counterclockwise direction when viewed from behind the swimming bacterium. Evidence for this conclusion was provided in two ways. First, the smooth-swimming bacteria moved in a clockwise direction at the lower surface of the chamber and in a counterclockwise direction at the upper surface. This is to be expected because of the frictional drag on bacteria whose flagella are rotating in a counterclockwise direction at the glass surface. Secondly, it was found that the flagella of some bacteria adhere to the surface of the glass slide and that the cell bodies of these bacteria rotate chiefly in a counterclockwise direction. The rotation of the free flagella would occur, therefore, in counterclockwise direction when viewed from the flagellum towards the body, as deduced by the antibody experiments of Silverman and Simon (9a).

Changes in direction are achieved by reversal of flagellar rotation. This is indicated, firstly, by the observation that the free-swimming, monotrichously flagellated bacteria at times reverse direction abruptly by simply backing up. An
almost 180° change occurs without tumbling of the bacteria (Fig. 1). Secondly, those Pseudomonas whose flagella stuck to the slide surface reversed rotation direction of the cell body spontaneously when left in a uniform medium. The bodies rotated chiefly in a counterclockwise direction but changed briefly to a clockwise rotation periodically. Thirdly, the reversal frequency is altered in gradients of attractant or repellent. Bacteria whose filaments were attached to the slide surface were found to oscillate between clockwise and counterclockwise rotation when exposed to a decrease in attractant or an increase in repellent. Some bacteria momentarily ceased rotation at frequent intervals. After exposure to an increase in attractant concentration, the bodies of cells rotated smoothly in a counterclockwise direction. These studies support the findings (1, 4a, 9a) that both spontaneous reversals and smooth rotation and reversals were induced by gradients of attractants and repellents. One difference is that Adler and co-workers (4a) observed that decreases in attractant caused essentially permanent reversal of rotation in E. coli, whereas we observed increased frequency of oscillation between clockwise and counterclockwise rotation in P. citronellolis.

The finding that P. citronellolis changes the forward direction swimming by briefly reversing the direction of flagellar rotation provides an explanation for the tumbling of the peritrichous bacteria. Hydrodynamic forces are believed to hold the flagella filaments in a bundle (11), although static forces might also be involved (6). A brief reversal of the flagella would disrupt the hydrodynamic forces and cause the flagella to fly apart. The asymmetry of the thrust by the unbundled flagella would cause tumbling. Support for this conclusion is provided by the observation that flagella fly apart during tumbling (6) and by the effect of light on Salmonella stuck to the glass slide by a flagellum. The bodies which are rotating freely in the medium reverse direction when illuminated by intense light (R. Macnab, personal communication). Tumbling is generated in free swimming Salmonella by the same pulses of intense light (6).

Thus, both the peritrichous and the monotrichous bacteria change direction by the same mechanism, a reversal of flagellar rotation. For the monoflagellated bacteria reversal causes the bacteria to “back up.” In the multiflagellated bundle, the reversal causes the flagella to fly apart and the bacterium to tumble. Sometimes Salmonella change direction abruptly without visible disruption of the flagella bundle; this may occur when one or more of the flagella briefly stop or rotate in a counterclockwise direction.

We are grateful to H. Berg, M. Simon, and J. Adler for sending us manuscripts in advance of publication.

This work was supported by grants from the Public Health Service (grant AM-GM-10765) and the National Science Foundation (grant GB7057).

**LITERATURE CITED**